JOURNAL OF DAIRY SCIENCE

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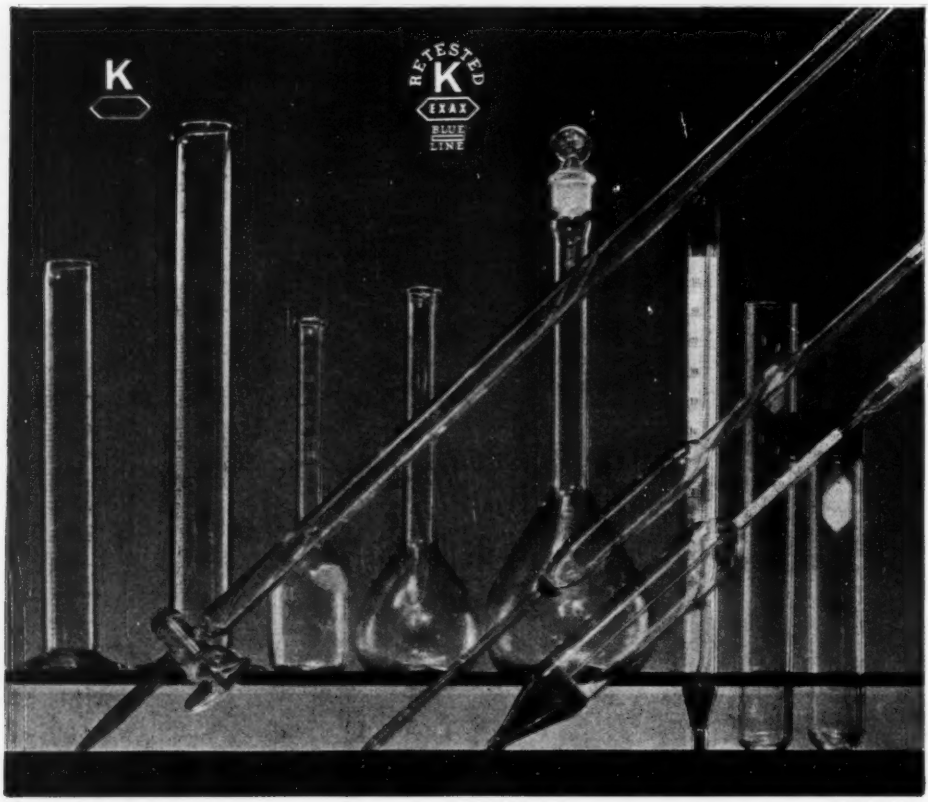

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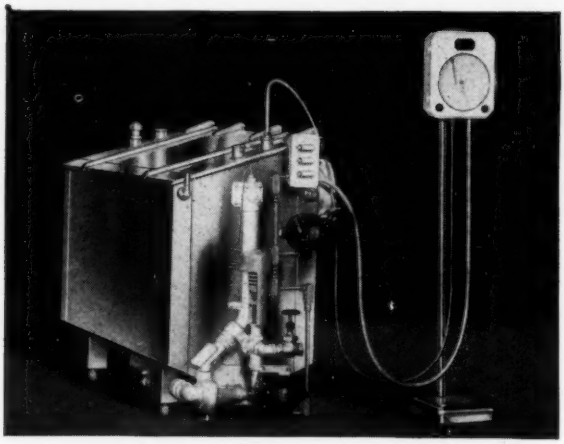

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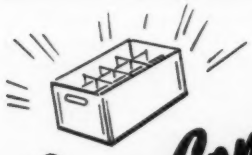

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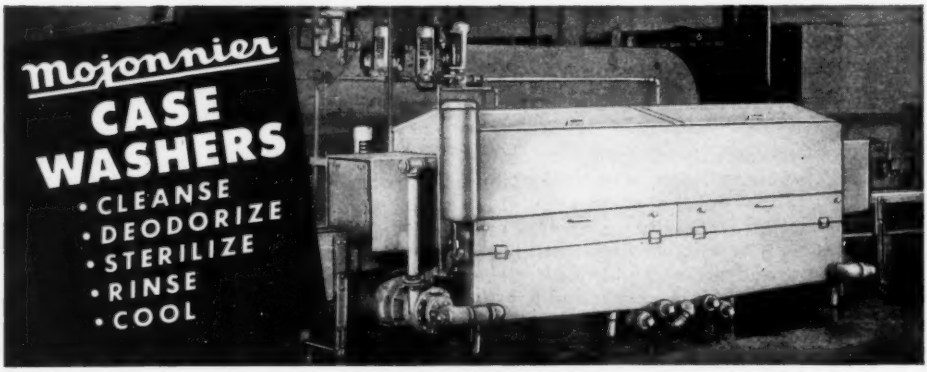

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JOURNAL OF DAIRY SCIENCE

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FACTORS MODIFYING THE EXCRETION OF FECAL ANDROGENS IN THE COW

R. B. CASADY, H. H. COLE AND G. H. HART

Division of Animal Husbandry, University of California, Davis

The function of androgens in the female is becoming recognized as one of considerable importance. Accumulating evidence indicates that androgens may play an important role in determining the normal processes associated with the estrous cycle. Salmon and Geist (23) have shown that androgens stimulate sexual libido in the human female and Cole *et al.* (2) report similar results with the ewe. Selye (24) states that androgens are a potent means of terminating menstruation and that testosterone produces mammary development in the female rat.

Since McGee (19) first extracted the male hormone from bull testes in 1927, investigations of androgen excretion have been confined almost entirely to the urine. In 1942 Riley and Hammond (22) showed, however, that the feces of dairy cows had considerable androgenic activity as determined by precocious comb and wattle growth in chicks given cow feces in the diet. There was also a retardation of gonadal development of the chicks. Fees of cows both with and without access to pasture contained the active principle in similar concentrations, a fact leading the authors to conclude that androgen is not derived from the diet. On the other hand, bull feces showed no evidence of androgen content. Turner (25 to 29) and Longwell and Gassner (8, 18), have confirmed and extended the findings of Riley and Hammond.

The purpose of the present study is to investigate factors modifying the fecal excretion of androgens in the cow, and to determine the possible relationship between the fecal androgen level and certain reproductive disorders in cattle.

EXPERIMENTAL

The animals used in these studies were Jersey and Holstein cows from the Division of Animal Husbandry herd with the exception of four Jerseys and one Guernsey with cystic ovaries purchased from nearby breeders. The method of assay used was that described by Riley and Hammond (22), with the exception that day-old White Leghorn cockerels were used in place of Rhode Island Red chicks and the feeding period was shortened to 14 days. White Leghorns were used because they were most readily available at the time the study was initiated. Dorfman (4) has shown since that the White Leghorn comb is 10 to

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20 times more responsive to androgen than the comb of the Rhode Island Red or Barred Rock. The shorter feeding period was instituted when it was noticed that the comb response at the end of 14 days was great enough to afford a reliable assay. Only cockerels were used, any pullets found at autopsy being discarded. Data from cockerels weighing less than 90 g. at autopsy were not included. It was intended to use ten or eleven chicks for each test but the number was reduced in some instances to seven by the limitations imposed by sex and size.

To rule out the possible effect of environment, a control group of chicks receiving no feces was run with each test. There was no evidence to indicate that changes in environment or in chicks used influenced the results.

The feces were collected in most instances in a clean container as they were dropped. In a few instances, part of the sample was obtained by artificial evacuation of the bowel; in a few others, samples were taken up from a clean concrete floor. The feces were dried in shallow pans in ovens at a temperature of 39 to 41° C. for 48 to 72 hours and then ground in a hammer mill before mixing with the chick ration.

The basal chick ration was a well-balanced diet furnished by the Poultry Division of the University of California.[1] The feces were mixed with the basal ration in the proportion of 10 per cent by weight and fed to day-old chicks. Fed in this proportion, the average consumption of feces was 18 g. per bird, with extremes of 15 to 21. There was as much variation in feces consumption among groups of chicks on the same feces sample as among groups on different samples. As differences between the means of only 2 of 18 repeat tests of given samples were significant, it is apparent that variations in feed consumption were not sufficient to be of great importance in the interpretation of the results. At the end of 14 days on feed, the combs were removed as described by Frank *et al.* (6). The testes were removed for weighing at the same time. The comb and testes were weighed separately on a torsion balance to the nearest 0.2 mg.

The chicks were kept in a Jamesway brooder with the temperature under the hover pad at 95 to 100° F. The room temperature was maintained at approximately 70° F.

EXPERIMENTAL RESULTS

Accuracy of the assay method. To test the accuracy of the androgen assay procedure, 18 of the feces samples were tested twice, using two groups of chicks at different times. During both feeding periods, conditions were duplicated as closely as possible with respect to ration, temperature, number of birds and length of feeding period. The mean comb weight of the repeat group was compared with that of the original assay group. Statistical analysis of the difference between the means showed that in 16 out of 18 compared assays the difference between the means was not significant at the 5 per cent level of probability

[1] The basal ration consisted of: fishmeal–16, meat scraps–16, soybean meal–56, alfalfa meal–20, salt–2, limestone and $MnSO_4$–4, fish oil–1, millrun–60, brewer's yeast–4, ground wheat–40, ground corn–173, and whey–8.

(table 1). Thus, this method of assay for fecal androgens is fairly reliable and significant differences in comb weights usually may be attributed to differences in the amount of androgens in the feces fed. At the present time no adequate explanation is available as to why significant differences were found between mean comb weights in two of the repeat tests.

Effect of diet on the fecal androgen level. Assuming that the 24-hour excretion of androgens is constant, greater androgenic activity per gram should be obtained from feces of cows on a low fiber diet with low total feces during the 24 hours than from cows on a roughage diet with a larger amount of feces

TABLE 1

Tests on the accuracy of fecal androgen assay by the chick comb growth method with feces fed as 10% of the diet[a]

Sample no.	Feces consumption per chick for the 2 tests:		Average comb weights per 100 g. body weight for the two tests:		Significance of difference between duplicate tests expressed as probability values[b]
	Original	Repeat	Original	Repeat	
	(*g.*)	(*g.*)	(*mg.*)	(*mg.*)	
2	18.2	15.3	71.2	71.7	> 0.50
6	19.7	16.7	105.6	100.8	> 0.50
3	16.9	17.6	322.2	290.5	> 0.50
15	18.4	17.7	252.7	276.3	> 0.50
19	18.2	19.4	228.2	214.2	> 0.50
18	17.0	18.4	272.9	268.4	> 0.50
5	17.2	16.7	271.7	246.1	> 0.50
8	16.6	16.4	279.4	246.5	> 0.50
31	18.9	19.7	250.2	245.8	> 0.50
32	18.5	.....[c]	429.3	409.7	> 0.50
4	17.9	15.2	333.5	284.4	0.37
12	18.1	17.4	325.2	282.6	0.37
17	18.5	18.1	244.3	212.6	0.36
13	15.9	19.5	110.0	158.7	0.20
33	17.3	.....[c]	403.2	321.0	0.20
30	15.3	19.3	159.9	237.2	0.20
28	15.2	20.6	119.9	254.1	0.05
16	19.0	17.8	200.9	125.4	< 0.01

[a] Duplicate tests were made on given samples with at least 1 week between tests. An average of 9 chicks were used for each test with extremes of 7 to 11.

[b] Probability values greater than 0.05 indicate that the difference between duplicate tests is not significant.

[c] Data not available.

voided. To test this hypothesis, two non-lactating cows were placed on a cement floor without bedding and fed alfalfa hay and straw in excess for a preliminary period of 3 days, at the end of which time a 24-hour collection of feces was made and an aliquot taken for assay. All hay and straw then was taken away and each cow was fed 5 lb. of rolled barley twice daily for a preliminary period of 3 days, at the end of which time another 24-hour collection of feces was made. The experiment was repeated using two other cows with a preliminary feeding period of 5 days for alfalfa hay alone and 4 days for grain. Again 24-hour collections of feces were made at the end of each feeding period and aliquots taken for assay. During all feeding periods the cows had access

TABLE 2

Effect of a roughage diet as compared to a grain diet on the fecal androgen level in cows as determined by the chick comb growth method[a]

Cow donor	Samples compared	Feces consumption per chick receiving feces of cows on:		Av. comb wt. per 100 g. body wt. with feces from cows receiving:		Significance of difference between means:	
		Rolled barley[b]	Roughage[c]	Rolled barley[b]	Roughage[c]	t	P
		(*g.*)	(*g.*)	(*mg.*)	(*mg.*)		
562	28 & 30	15.2	15.3	119.9	159.9	1.15	> 0.2
562	28 & 30	20.6	19.3	254.1	237.2	0.22	> 0.5
E-59	27 & 29	17.3	16.4	62.2	117.2	3.93	< 0.01
757	45 & 46	19.2	19.4	187.2	461.0	4.99	< 0.01
821	43 & 44	20.5	18.0	99.7	534.1	6.45	< 0.01

[a] The dried feces were fed as 10% of the chick ration for 14 days. An average of 9 chicks were used per test.
[b] 5 lb. twice daily.
[c] Cows 562 and E-59 received alfalfa hay and straw in excess.
Cows 757 and 821 each received 10 lb. of alfalfa hay twice daily.

to no other food, and water and salt were furnished *ad libitum*. Contrary to the hypothesis mentioned, the feces of the cows on a diet of roughage showed greater androgenic activity than feces of cows on the grain diet in three of four instances (table 2). The differences in comb weight produced by the feces of cow 562 on the two diets are not significant at the 5 per cent level of probability, but those for cows E-59, 757 and 821 are highly significant at the 1 per cent level. No explanation is available at present to account for the difference in comb weight between the original and repeat tests on each diet in the case of cow 562. On the hay diet, the total amounts of feces excreted during the 24-hour period were 2.6, 3.8, 3.8 and 4.3 times greater than on grain alone for cows 562, E-59, 757 and 821, respectively (table 3). Thus, considering the greater androgen content per gram of feces in three out of four cases while on the hay diet, it is apparent that the total fecal androgen excretion is more than three times that on the grain diet. These data clearly indicate that diet has a promi-

TABLE 3

Influence of diet on 24-hour excretion of feces

Cow donor	Dry weight of feces on:		Per cent increase
	Rolled barley[a]	Roughage[b]	
	(*g.*)	(*g.*)	
562	1350	3505	260.0
E-59	1145	4320	380.0
757	815	3140	380.0
821	795	3420	430.0

[a] 5 lb. twice daily.
[b] Cows 562 and E-59 received alfalfa hay and straw in excess.
Cows 757 and 821 each received 10 lb. alfalfa hay twice daily.

nent role in determining the fecal androgen level. This effect of diet must be considered in comparative assays. All comparative assays made during the course of this study were made on cows receiving similar diets, and the role of the diet is not considered to have influenced the results to any appreciable degree.

Androgen levels in normal cows. To establish a basis for comparison, assays were made of feces of normal dairy cows in various physiological states. Feces of non-pregnant lactating, pregnant lactating and pregnant non-lactating cows produced comb weights 300 to 400 per cent greater than the controls, at the same time suppressing testicular development to 50 to 60 per cent of that of the controls (table 4). The data fail to show a clear-cut difference in androgenic

TABLE 4

Androgen in the feces of normal dairy cows as determined by the chick comb growth response[a]

Cow donor	Condition of donor	Sample no.	No. of chicks	Feces consumption per chick	Av. body weight	Av. wt. of testes	Av. comb wt. per 100 g. body wt.
				(*g.*)	(*g.*)	(*mg.*)	(*g.*)
None	Controls	.....	57	.....	128.5	38.4	73.8
904	Non-pregnant lactating	3	7	16.9	116.7	18.7	322.2
		3	11	17.6	107.4	14.9	290.5
1092	Pregnant lactating[b]	4	10	17.9	112.9	16.7	333.5
		4	9	15.2	103.4	14.7	284.4
1093	Pregnant lactating[b]	5	7	17.2	113.7	18.7	271.7
		5	8	16.7	100.7	19.2	246.1
885	Pregnant non-lactating[c]	8	11	16.6	110.7	21.9	279.4
		8	8	16.4	113.6	17.4	246.5
907	Pregnant non-lactating[c]	9	8	..... [d]	104.0	17.3	241.5
		11	11	17.7	115.6	18.8	385.2
897	Pregnant non-lactating[c]	12	9	18.1	115.5	18.1	325.2
		12	10	17.4	112.4	16.8	282.6

[a] The feces were fed to chicks for 14 days as 10% of the ration. Sample numbers indicate where repeat tests were conducted

[b] Cows in first 2 months of gestation.

[c] Cows in last month of gestation.

[d] Data not available.

content of the feces in the different groups. Turner (28) reported that the androgenic activity was high during the first month of lactation but a later report (29) did not substantiate this. Gassner and Longwell (8) report that the androgenic titer drops to zero at parturition. Several reports (8, 18, 28, 29) indicate that the excretion is high shortly before parturition but none specifically states that statistically significant increases were found. In our series, two of three samples collected in late pregnancy did not vary significantly from the mean of all samples collected from normal non-pregnant or early pregnant cows.

Androgen levels in cystic-ovary cows. The etiology of cystic ovaries in cows is as yet unknown. This pathological condition has been studied by several

investigators since the turn of the century (1, 7, 10, 11, 20, 30) but studies on hormonal levels in these animals are not available.

Six cows with a cystic condition of the ovaries were studied during the course of this investigation. A brief history of each case follows:

Cow 1. Jersey, 7 years of age, lactating approximately 2 years. Placed under observation June 22, 1947. Examination on that date disclosed a cyst on the left ovary double the size of a walnut, no cyst on the right ovary. Previous history of frequent mounting and molesting other cows in the herd. Evidence of heat once in 2.5 months under our observation.

Cow D-41. Jersey. Last calf September, 1945. Cyst first discovered November, 1946, shortly after abortion. Cyst expressed for the last time December, 1946. Placed under observation October 28, 1947 and examination revealed the right ovary the size of a small orange, a little larger than the left. Previous history of lowing like a bull and riding all cows available. Used to determine cows in heat. Tail head not significantly raised. During study infrequent riding of companion cow was observed. In heat December 19, 1947. Examination December 20th disclosed a definite cyst, 2×2.5 inches, on the left ovary. Right ovary was normal. Autopsy at end of study showed the right ovary to be 1×1.75 inches with one follicular cyst; the left, 1.75×2 inches with three follicular cysts. No corpora lutea were present.

Cow E-59. Jersey. Last calf June 6, 1946. Obtained for study March 1, 1948. Previous history of nymphomania symptoms, frequent estrous period and breedings, but no conceptions, bellowing, high at the tail head, riding of all cows in heat, pawing and nervousness. No heats observed from March to June, 1948. Examination February 23, 1948, showed the right ovary to be the size of a small hen's egg with a cyst 1 inch in diameter. The left ovary was the size of a large English walnut but had a smooth surface. The ovaries regressed until April 20, when examination showed one cystic follicle on the right ovary. Last examination in June, when the right ovary was the size of an English walnut and had one or more large follicles and the left ovary was small with no palpable follicles or corpora lutea. Cow anaphrodisiac since February, 1948, but still repeatedly mounts cows in heat, is high at the tail head and bellows like a bull.

Cow 216. Last calf June, 1947. Bred every 3 weeks on occurrence of heat between July, 1947, and January, 1948, with no conceptions resulting. No external cystic symptoms except for a slightly raised tail head. Previous history of mounting cows in heat a great deal. Examination in February, 1948, revealed the right ovary inactive, the left ovary half the size of a hen's egg and definitely cystic. Examination again in March disclosed no cysts on either ovary. In heat March 1, 1948, and bred March 2.

Cow 849. Jersey. Last calf December 11, 1947. Bred four times within a 2-month period since her last calf. History of cyst first being noticed March 24, 1948, on the right ovary; left ovary normal. Placed under observation May 11, 1948. Previous observations indicate she frequently was in heat, riding other cows and standing to be ridden. Between May 11 and June 6, she was in heat every 7 to 9 days and exhibited nymphomania symptoms of being very rabid, riding and being ridden almost constantly while in heat. On March 11, the cyst on the right ovary was 0.75 inch in diameter and quite hard. Examination June 3 revealed both ovaries enlarged, a little larger than an English walnut. Injected with 1000 I.U. equine gonadotrophin June 4, and by June 9 the ovaries were smaller, although the left was still about the size of an English walnut, the right ovary being smaller and flatter with a corpus luteum on one end. In heat June 6, but was not observed in heat again up to June 29, although she still mounted other cows in heat. Estrus noted again July 17, July 20 and July 22.

Cow 758. Jersey. Last calf September 3, 1947. Bred five times between September, 1947, and January, 1948. History of displaying nymphomaniac symptoms. In heat often, every 4 to 5 days. Showed morphological characteristics of a high tail head, relaxed pelvic ligament, and tendency toward coarseness in the shoulders. Did not develop the throaty bawling of a bull. Examination March 23 and March 25, 1948, disclosed the left ovary slightly enlarged with a couple of small cysts present with the right ovary apparently normal.

Feces of the six cows were collected and assayed for androgenic activity (table 5). Feces of cows 1 and D-41 produced comb weights which indicated very little excretion of androgens, while those of the remaining cows showed significant androgen content, although two of them were below that of the normal cows. The results suggest a decrease in fecal androgens in cows with cystic ovaries, although results on cows 849 and 758 might be considered as being in the normal range. As may be noticed in the table, there is an apparent correlation between the fecal androgen level and the length of time since calving. The lowered androgen excretion in cystic-ovary cows with masculine charac-

TABLE 5

Androgen in the feces of cystic-ovary cows as determined by the chick comb-growth response[a]

Donor	Months since last calf	Sample no.	No. of chicks	Av. body weight	Av. wt. of testes	Av. comb per 100 g. body weight	Feces consumption per chick
				(*g.*)	(*mg.*)	(*mg.*)	(*g.*)
None (controls)[b]	.....	.....	13	329.1	86.8	402.4	.....
Cow no. 1[b]	24	1	15	258.1	51.8	381.4	.....[c]
		1	14	249.2	46.2	543.1	.....[c]
None (controls)	.....	.....	161	128.8	40.2	80.1	.....
D-41	26	2	9	115.5	28.5	71.2	18.2
		2	10	128.9	34.0	71.7	15.3
		6	9	109.8	30.5	105.6	19.7
		6	8	120.0	26.6	100.8	16.7
E-59	33	17	10	123.4	25.1	244.3	18.5
		17	11	125.1	19.6	212.6	18.1
		20	10	107.5	24.6	104.0	16.9
216	8	13	9	112.1	21.8	110.0	15.9
		13	10	129.5	30.3	158.7	19.5
849	5	36	9	137.2	29.2	268.2	20.1
758	6	23	8	111.0	20.0	234.9	16.2

[a] The feces were fed to chicks for 14 days as 10% of the ration. Sample numbers indicate where repeat tests were conducted.

[b] Results over a 28-day feeding period.

[c] Data not available.

teristics and behavior is analogous to the low androgen excretion in the bull as reported by Riley and Hammond (22) and Turner (27). The significance of this decreased excretion of fecal androgens in the male and masculine-like female is not apparent.

Effect of ovariectomy on androgen excretion. It has been demonstrated that the ovary may secrete androgens under certain conditions (3, 12, 13, 14, 15, 17), but to what extent it is involved in the normal production of androgens has not been determined. To investigate the role which the ovary plays as a source of fecal androgens in the cow, bilateral ovariectomy was performed on cow 883. Feces were collected for assay preceding the operation and on the 4th, 10th and 39th days post-operative. The results are shown in table 6. Ovariectomy did not produce a decrease in androgen excretion. In fact, there is an apparent

increase in excretion on the 10th and 39th days post-operative. Failure of ovariectomy to decrease androgen excretion coincides with the results obtained in the human female (9, 16). Dorfman *et al.* (5) observed a decrease in urinary androgen excretion after ovariectomy in one female monkey, and no change in excretion after ovariectomy in a second female. If the ovary is directly concerned with androgen excretion, the results of ovariectomy may be explained by assuming that the stimulating influence of stress on the adrenal

TABLE 6

Effect of ovariectomy and ovarian stimulation with equine gonadotrophin on the fecal androgen level in cows as evidenced by chick comb growth[a]

Cow donor	Condition of donor	Elapsed time	Sample no.	No. of chicks	Feces consumption per chick	Av. body weight	Av. comb wt. per 100 g. body weight
		(*days*)			(*g.*)	(*g.*)	(*mg.*)
883	Pre-operative	18–26 (composite)	10	9	18.4	113.7	249.3
			10	9	17.9	114.8	126.5
			10	9	17.7	125.8	173.7
	Post-operative	4	16	8	19.0	135.6	200.9
			16	11	17.8	113.7	125.4
		10	18	10	17.0	109.7	272.9
			18	10	18.4	121.9	268.4
		39	21	9	16.4	109.0	239.4
562	Pre-injection	1	37	10	19.8	129.2	156.5
	Post-injection[b]	4	38	7	19.7	133.9	190.2
		10	39	8	19.1	125.8	240.3
	Pre-injection	49	7	10	17.6	118.7	144.9
		13	14	7	17.1	114.4	178.7
	Post-injection[c]	4	15	9	18.4	120.8	252.7
			15	8	17.7	117.9	276.3
		10	19	9	18.2	114.3	228.2
			19	11	19.4	125.7	214.2
		39	22	8	16.2	110.1	204.9
E-59	Pre-injection	1	40	11	.....[d]	127.3	325.2
	Post-injection[b]	4	41	9	.....[d]	126.0	161.8
		10	42	9	.....[d]	136.5	193.7

[a] The feces were fed as 10% of the chick ration for 14 days. Sample numbers indicate where repeat tests were conducted.
[b] Injected with 1400 I.U Equine Gonadotrophin.
[c] Injected with 3000 I.U. Equine Gonadotrophin.
[d] Data not available.

masked the decrease resulting from ovariectomy, since Pincus (21) has shown that stress causes an increase in ketosteroid excretion.

Effect of equine gonadotrophin on androgen excretion. To investigate further the role which the ovary may play as a source of fecal androgens, cow 562 was injected with 3000 I.U. equine gonadotrophin and the feces collected for assay on the 4th, 10th and 39th days following injection to observe the effects of ovarian stimulation. Subsequently, cows 562 and E-59 were injected with

1400 I.U. equine gonadotrophin and feces collected on the 4th and 10th days following injection. Table 6 shows the results obtained from fecal assays after ovarian stimulation. It may be noted that increased excretion of fecal androgens was obtained in the two experiments on cow 562 and decreased excretion in the experiment on cow E-59. The long lasting effect in cow 562 (39 days) may be due to the great sensitivity of this cow to gonadotrophins. Her ovaries exhibited great follicular development, increasing in size to that of an orange and not regressing for a period of 2 months. The results in cow E-59 may be due to lack of ovarian stimulation. Periodic manual examination disclosed that her ovaries did not respond with the follicular development and hypertrophy as observed with cow 562. These data, considered in the light of the previous ovariectomy experiment, suggest that the ovary does not influence directly the fecal

TABLE 7

Effect of unilateral adrenalectomy on the fecal androgen level of cow 883 as determined by by the chick comb-growth response[a]

Condition of donor	Elapsed time	No. of chicks	Av. body weight	Av. comb. wt. per 100 g. body weight	Feces consumption per chick
	(*days*)		(*g.*)	(*mg.*)	(*g.*)
Ovariectomized pre-adrenalectomy		8	124.1	250.2	18.9
		9[b]	126.5	245.8	19.7
Post-adrenalectomy	3	11	114.1	429.3	18.5
		10[b]	135.6	409.7	.....[c]
	7	8	112.8	403.2	17.3
		10[b]	132.7	321.0	.....[c]
	11	11	114.5	318.9	18.2
	14	9	127.8	468.3	19.8

[a] The feces were fed as 10% of the chick ration for 14 days.
[b] Repeat test on same sample of feces.
[c] Data not available.

androgen level appreciably in the cow but may exert an indirect effect through the medium of some other gland.

Role of the adrenal in androgen excretion. Since the adrenals have been shown quite conclusively to be one site of androgen formation in various species, the possibility of their being concerned with androgen excretion in the cow seems very likely. With that in mind, a cow was adrenalectomized unilaterally and feces collected for assay before the operation and 3, 7, 11 and 14 days postoperative. The cow used for this experiment was cow 883 which had been ovariectomized bilaterally 9 weeks previously. After the removal of one adrenal, there was increased androgen activity of the feces during the 2-week period the feces were collected (table 7). One might expect that the removal of one adrenal would result in a decrease in androgen until such time as the other adrenal hypertrophied and compensated for the loss. Dorfman *et al.* (5) observed a decrease in androgen excretion to one-third the normal level after removal of gonads and both adrenals in male and female monkeys. Adrenalec-

tomy in the cow is a hazardous and difficult procedure, however, resulting in considerable stress and post-operative discomfort. Very little food or water was consumed by the cow for approximately 72 hours after the operation, and defecations were extremely meager. Furthermore, severe infection was found at the site of the removed adrenal at the time of autopsy 26 days after the operation. One may assume, therefore, that, as a result of stress, the remaining adrenal secreted at a greater rate than did both adrenals preceding the operation. The fact that a marked change in fecal androgens occurred following adrenalectomy in an ovariectomized cow provides strong presumptive evidence that the adrenals play a prominent role in androgen production in this species.

DISCUSSION

One of the first questions confronting one attempting to study the factors modifying the excretion of a given hormone is the accuracy of the assay test to be employed. The data given in table 1 indicate that in most instances a given result can be duplicated with considerable precision. However, in 2 of 18 tests there were statistically significant differences in the mean comb weights of duplicate tests on the same sample. This means that in a few instances there have been variables in the procedure or in the test animals which have not been recognized. This fact serves as a warning in the interpretation of minor differences.

That the nature of the diet has a profound effect upon the 24-hour excretion of fecal androgen is indisputable. In three of five tests the fecal androgen per gram of feces was distinctly higher when cows were fed roughage alone than when they were fed concentrates alone (table 2). In addition, the total feces per 24-hour period was approximately three times greater on roughages than on grain (table 3). Until the specific androgen in cow feces is known, attempts to express androgen excretion in absolute terms are of doubtful value. Nonetheless, the response of the chick comb to dehydroisoandrosterone[2] was determined during the course of this study. When placed in the chick ration using the same strain of chicks, 4 mg. of this androgen per bird produced a comb weight (average of 10 birds) of 170 mg., and 8 mg. of the hormone, an average comb weight of 318 mg. The responses to the feces of cow 757 (table 2) come closest to simulating these responses. The amount of feces consumed per bird and the 24-hour excretion of feces are known. Assuming that the response to the feces of cow 757 on rolled barley, 187 mg. comb weight, is equivalent to the response to 4 mg. of dehydroisoandrosterone, and further that the response to the feces of cow 757 on alfalfa hay, 461 mg. comb weight, is equivalent to the response to 8 mg. of the hormone, one is able to calculate excretion in terms of dehydroisoandrosterone. The calculation discloses that an equivalent of 212 mg. of dehydroisoandrosterone was excreted daily by cow 757 on rolled barley and 1,286 mg. on alfalfa hay. In other words, the daily androgen excretion was approximately six times greater on roughage than on grain.

[2] Kindly supplied by Schering Corp., Bloomfield, N. J.

Further studies are necessary before one could speculate on the cause of this difference.

The consistency with which responses of 200 mg. or greater in comb weights were obtained when chicks were fed feces from normal animals (table 4) indicates that lower values may have diagnostic significance. As is seen in table 5, a low androgenic response usually is obtained from cows with cystic ovaries of long standing.

The results clearly show that there is no fall in the fecal androgen level following ovariectomy. Too much emphasis should not be put on the rise observed without further experiments. The significance of the finding that in two trials a distinct rise was obtained after treatment of cow 562 with equine gonadotrophin is tempered by the occurrence of a distinct drop in a trial with cow E-59. The fact that a distinct increase in ovary size was found by rectal palpation in the first animal and no detectable ovarian change in the second may furnish a clue for interpretation.

The distinct rise in androgen excretion obtained by unilateral adrenalectomy scarcely is the result expected, but, nevertheless, it does indicate that the adrenal may be involved in determining the amount of fecal androgen in the cow.

SUMMARY

Studies were made on the fecal androgen excretion by dairy cows in various physiological and pathological states.

The accuracy of the assay method was determined with duplicate tests on 18 samples. Sixteen of the 18 tests showed no statistical difference between the mean comb weights of duplicate assays on the same sample.

In four trials, diet was shown to have a marked effect on the androgenic activity of cow feces; the 24-hour excretion of androgens on a roughage diet (alfalfa hay and straw, or alfalfa hay alone) was three to four times greater than on a diet of concentrates alone. The androgenic activity per gram of feces and the total feces excreted in 24 hours both were greater on roughage than on concentrates.

The feces of normal cows, incorporated as 10 per cent of a chick diet, produced an average comb weight per 100 g. body weight of 295.2 as compared to an average comb weight of 80.1 mg. for control chicks receiving no feces. In the small number of animals studied, there was no indication that the fecal androgen level was influenced significantly by lactation or stage of gestation.

The feces of cows with cystic ovaries contained less androgenic activity than the feces of normal animals in four of six cases. In fact, in two cases no evidence of androgen could be detected by the method employed. Thus, it appears that in certain reproductive abnormalities, the fecal androgen level is decreased.

Bilateral ovariectomy of a cow caused an apparent increased androgen excretion. Feces collected 4, 10 and 39 days following ovariectomy produced average comb weights per 100 g. body weight of 157.2, 270.6 and 239.4 mg.,

respectively, as compared to the average comb weight of 183.2 mg. produced by feces collected before the operation.

Ovarian stimulation with equine gonadotrophin produced increased excretion of androgen in two experiments and decreased excretion in one experiment.

Unilateral adrenalectomy of an ovariectomized cow was followed by an increase in androgen excretion. Feces collected 3, 7, 11 and 14 days post-operative produced average comb weights per 100 g. body weight of 419.9, 357.5, 318.9 and 468.3 mg., respectively, as compared to 248.0 mg. for feces collected pre-operatively. As the gonads were absent, a plausible explanation of the results obtained is that the remaining adrenal was stimulated to increased secretory activity.

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SOME EFFECTS OF FEEDING THYROPROTEIN TO DAIRY COWS DURING THEIR FIRST LACTATION

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During the past several years various investigators have demonstrated that feeding thyroprotein, an iodinated casein, to producing dairy cows results in increased milk production. Reineke and Turner (16) were the first American workers in this field. The results of the early work have been verified and expanded at several experiment stations (1, 3, 6, 10, 13, 15, 25). Seath *et al.* (19) have studied the effect of feeding thyroprotein in a warm climate. The largest experiment was one carried out by Blaxter (4) in England which involved the use of 1,008 cows in farm herds. This investigator has been studying recently the possibility that thyroprotein feeding may have practical application in England if the thyroprotein is fed when milk production is at a seasonal low point during the latter part of the winter, a period of about 2 months (5). The changes that might occur in the composition of the blood and/or milk as a result of feeding thyroprotein to the dairy cow also have been studied (1, 6, 10, 12, 20, 26). A review of a non-scientific nature on feeding of thyroprotein to dairy cattle appeared in the 1943–47 Yearbook of Agriculture (14).

Most of these experiments have been for short periods and, therefore, the data are not adequate to guide the dairyman in feeding thyroprotein to cows over long periods of time. Also, there are no data concerning the effects of feeding thyroprotein for long periods and/or for several lactations on the health and lifetime performance of cows. Therefore, an experiment was set up by the Bureau of Dairy Industry to study the long-time effects of feeding thyroprotein on the health and reproduction of dairy cattle. Some interesting observations have been made from the standpoint of milk production and the results observed during the first lactation are reported here.

EXPERIMENTAL

Eleven first-calf heifers were fed thyroprotein from approximately the 50th day postpartum until 90 days before the next expected parturition. This amounted to an average of 301 days (range 240 to 380) during which the cows received the drug. A group of three control cows (group 1) received the same feed and treatment except that they were not fed thyroprotein.

All cows were fed 7.5 lb. alfalfa and 7.5 lb. timothy hay and 15.0 lb. of corn silage per 1000 lb. of body weight. This roughage allowance was calculated in early lactation and maintained at that level during the lactation. The remainder of the nutrient allotment of each cow consisted of a 17 per cent protein grain mixture of wheat bran, 40 parts; ground yellow corn, 40 parts; linseed meal, 20 parts; and salt (non-iodized), 1 part.

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Three levels of thyroprotein feeding were used with cows of group 2. Cows H-268, H-269, J-488 and J-489 were fed 1.5 g. per 100 lb. of body weight Cows H-262, J-487, J-490, J-496 and H-266 were fed 1.0 g. per 100 lb. and cows J-604 and H-272 were fed 0.6 g. per 100 lb. The letter H before the number indicates the cow was of the Holstein breed; likewise, J indicates Jersey. During the experiment the amount of thyroprotein was adjusted every 10 days according to the cow's body weight. The weighed amount of thyroprotein was mixed with the daily grain alowance.

The cows receiving thyroprotein (group 2) were divided into three subgroups on the basis of total digestible nutrient (T.D.N.) intake. Group 2-A was fed the maximum Morrison requirements based on production and maintenance, as was group 1 (controls). The amount fed to group 2-B was increased after mid-lactation from 100 per cent to either 125 or 150 per cent of requirements and group 2-C was fed 125 per cent of the Morrison requirements throughout the period when thyroprotein was fed. This extra amount of nutrients was not always consumed during the early part of lactation. However, by the 100–150th day of lactation the cows readily consumed the 125 per cent and even 150 per cent when it was offered. The extra allowance of nutrients was fed only during the period thyroprotein was being fed.

Adjustments in the amount of grain fed were made at 10-day intervals and were based on the cow's body weight and milk production at similar intervals. In late lactation the grain allowance was not decreased below a minimum of 0.5 lb. per 100 lb. of body weight. This allowance was continued during the dry period. Thus toward the end of the lactation of groups 1 and 2-A and during the dry period of all cows the T.D.N. consumption was somewhat larger than requirements. Daily feed records of amounts fed and refused served as a basis for calculating T.D.N. consumption of each cow. Composite milk samples were obtained for butterfat test (Babcock) on the 5th and 6th days of each 10-day period.

RESULTS

Initial response and body weight changes. When thyroprotein was fed, all 11 cows responded with an increase in F.C.M. production. The degree of response was variable, some cows showing a considerable increase while others gave a negligible increase. Low responses were given by cows H-269 and J-604, but the increase was transient and too small to show when the production was averaged by 30-day periods. The low rate of thyroprotein feeding (0.6 g. per hundred-weight) to cow J-604 may have been one reason for the small response of this animal. The greatest response was given by cow J-489. This cow's average daily F.C.M. production for the month after thyroprotein feeding was initiated was 8.5 lb. (32 per cent) above her pre-experimental level.

The average daily milk production for the month previous to feeding thyroprotein and the first, second, third and fourth months after are as follows: Groups 2-A and B: 33.6, 36.6, 33.0, 28.5 and 24.3; Group 2-C: 34.3, 36.5, 37.5, 35.6 and 33.3. Corresponding figures for the control cows were 39.0, 35.6,

31.1, 27.9 and 25.9. In figure 1 the average milk production of these groups is graphically presented by 30-day intervals expressed as a percentage of the pre-experimental month.

The cows fed thyroprotein increased an average of 8 per cent (2.8 lb.) in daily milk production during the first month after thyroprotein feeding was started as compared to the pre-experimental month. However, by the fourth and fifth months after thyroprotein feeding was started, the production of the cows in groups 2-A and 2-B (both receiving T.D.N. at 100 per cent of requirement to this time) had decreased on a percentage basis to or below the production of the control cows. Thus after the initial increase in milk production the rate of decline was faster for cows fed thyroprotein and T.D.N. at requirement than it was for the control cows. A very different type of response was ob-

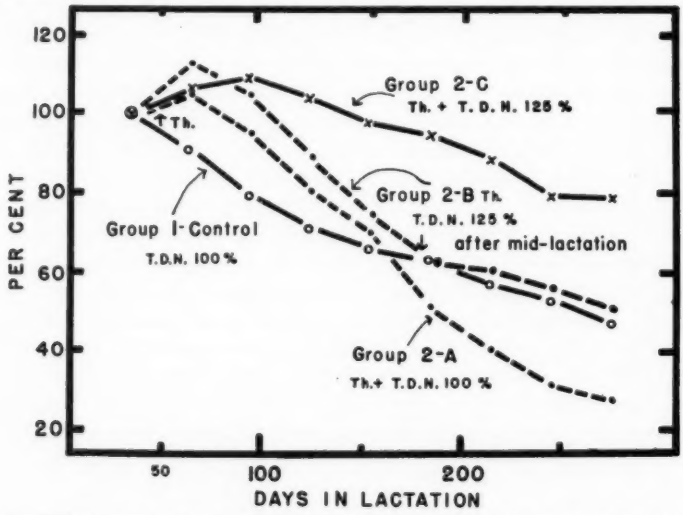

FIG. 1. Effects of feeding thyroprotein with different T.D.N. intakes on the average milk production expressed as a percentage of the pre-experimental month.

tained with the cows in group 2-C, which were given thyroprotein with extra T.D.N. at 50 days postpartum. The peak of response when extra nutrients were fed did not occur until the second or third month after thyroprotein feeding was begun, whereas cows receiving only 100 per cent of their T.D.N. requirement reached their peak within the first month after thyroprotein feeding was begun. Groups 2-A and B, after 4 to 5 months of their regime, had declined in milk production to about 65 per cent of their pre-experimental level, while at the same time the cows that received extra nutrients were producing at about 96 per cent of their pre-experimental level. Thus milk production, after being stimulated by thyroprotein, did not decline as rapidly in the group receiving thyroprotein and extra nutrients as it did in the group receiving thyroprotein and nutrients at 100 per cent of requirement.

When thyroprotein was fed to cows receiving nutrients at 100 per cent of their requirement (groups 2-A and 2-B) large losses in body weight occurred. These losses continued until late in the lactation. Over a corresponding lactation segment the control cows usually gained weight or had small decreases. The average body weight changes from the 50th to the 170th day were as follows: Group 1, a gain of 11 lb.; groups 2-A and B, a decrease of 78 lb. and group 2-C, a gain of 5 lb. The animals fed thyroprotein and extra nutrients from the 50th day had normal body weight changes (group 2-C). Graves (9) reported that well-fed first-calf heifers did not lose weight but usually made small gains after the first month postpartum. This was observed in the control and extra nutrient groups. Thus the feeding of extra nutrients with the thyroprotein prevented the large weight losses noted in animals receiving thyroprotein and nutrients at 100 per cent of requirement.

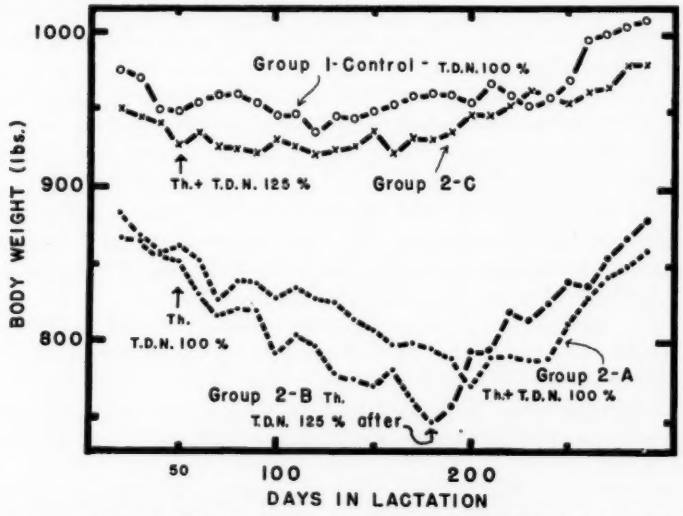

FIG. 2. Effects of feeding thyroprotein with different T.D.N. intakes on the average body weights of the experimental animals.

Effect of increasing T.D.N. intake at mid-lactation. An attempt was made to retard the rapid decreases in body weight and milk production of some cows (group 2-B) that had been receiving thyroprotein and T.D.N. at requirement. This was done by increasing the nutrient intake to 125 or 150 per cent of requirement by feeding additional grain after mid-lactation (at approximately 180 days) to four of the thyroprotein-fed cows. The effects of this treatment on their milk production, body weight and pulse rates can be observed by comparing group 2-A with group 2-B in figures 1, 2 and 3.

Figure 1 shows that the extra nutrients fed to group 2-B beginning at mid-lactation checked the abnormally rapid rate of decline in milk production and from this time on the persistency of this group was comparable to that of nor-

mally-fed cows. However, group 2-A, not fed extra T.D.N., continued to decline in milk production at an abnormally rapid rate. The decrease in daily F.C.M. production of group 2-B during the 2 months following the feeding of extra nutrients averaged only 2.0 lb. A corresponding figure for this segment of lactation of the control group was 3.7 lb., while for group 2-A, not fed extra nutrients, it was 6.4 lb. Throughout the entire lactation the persistency of milk production was excellent for all three first-lactation heifers that received thyroprotein and extra nutrients beginning at 50 days postpartum (group 2-C).

The cows receiving no extra feed (group 2-A) actually weighed less at 240 days in lactation than they did at 180 days (fig. 2). However, the cows in group 2-B, fed extra T.D.N., had gained 75 lb. during this period. The control animals gained an average of 21 lb. Thus the cows that had received thyroprotein for about 120 days and nutrients at 100 per cent of their requirement responded with marked gains in body weight when given extra feed. The body weight of those that did not receive this extra feed continued on a downward trend. However, after the 240th day, rapid increases in body weight occurred in group 2-A due to the fact that milk production had reached a low level and their minimum grain allowance was more than adequate to meet their requirement. When the extra T.D.N. was fed to the cows at mid-lactation, the heart rate showed a marked increase. Variations in heart rate are discussed later in the text. Thus, cows that are receiving thyroprotein and nutrients at 100 per cent of their requirement will respond with increased body weight gains, increased persistency in milk production and increased heart rates when given nutrients at 125 per cent of requirement.

When thyroprotein was removed from the ration at 90 days before the next expected parturition the body weight of all cows increased very rapidly. The increase in body weight averaged about 70 lb. during the first 20 days after the removal of thyroprotein. This rapid gain in body weight and the general appearance of the animal suggests a possible change in water retention when thyroprotein is fed.

The 305-day production records of 14 first-lactation heifers are presented in table 1. They are divided into one control group and three experimental groups that received thyroprotein beginning at about the 50th day of lactation and continued throughout the lactation period. The T.D.N. consumed varied in the three experimental groups.

No definite conclusions can be made concerning total production as affected by the experimental procedures involved because of the small number of animals and the inability to balance inherited productive capacity among the groups. The indications are that the total production for the lactation of cows fed thyroprotein with T.D.N. at requirement would be no greater than cows fed T.D.N. at requirement without thyroprotein. However, the total production of cows H-269, J-488, and J-604 was very low. The conclusion seems justified that poor producing cows cannot be changed into good producers by feeding thyroprotein.

Comparative efficiency of milk production. The comparative efficiency of milk production by the control group and thyroprotein-fed groups is presented in table 2. The lactation segment from the day thyroprotein feeding was started to the 305th day of lactation was used for this comparison. Two animals (J-604 and H-272) that were fed thyroprotein at the low rate were excluded from these calculations.

The F.C.M. produced, the T.D.N. consumed and weight gained during this period were obtained from the records. The T.D.N. assigned to maintenance during this period was calculated by summation of 10-day maintenance requirements based on body weight and Morrison's standard. The T.D.N. for weight gain was calculated by using the figure 3.53 lb. T.D.N. per lb. gain (17).

TABLE 1

305-day production records of 11 cows that have finished their first lactations on the thyroprotein experiment

Cow no. and breed	Rate of thyroprotein feeding	Milk	Butterfat	F.C.M.
	(g./100 lb.)	*(lb.)*	*(lb.)*	*(lb.)*
Group 1. Control cows (fed according to Morrison's maximum standard).				
J-491	0	5,499	286	6,488
J-485	0	5,930	342	7,505
H-263	0	9,625	413	10,007
Group 2-A. Thyroprotein cows (fed according to standard).				
J-604	0.6	5,181	255	5,897
H-268	1.5	6,853	283	6,979
H-269	1.5	4,693	199	4,863
J-487	1.0	6,514	346	7,804
Group 2-B. Thyroprotein cows (fed 125% of standard last 120 days).				
H-262	1.0	12,017	501	12,330
J-488	1.5	5,297	265	6,106
J-489	1.5	5,778	344	7,474
J-490	1.0	7,020	361	8,226
Group 2-C. Thyroprotein cows (fed 125% of standard during time fed thyroprotein).				
J-496	1.0	7,683	425	9,454
H-266	1.0	10,445	464	11,152
H-272	0.6	9,055	347	8,823

The T.D.N. consumed per 100 lb. of F.C.M. produced was calculated. A similar figure which excluded the nutrients used for weight gain was obtained when the T.D.N. assigned to weight gain was subtracted from the total T.D.N. intake. These two figures were used as a measure of gross efficiency for milk production. The net T.D.N. consumed per 100 lb. of 4 per cent F.C.M. produced were calculated by deducting T.D.N. assigned to maintenance and weight gain from the total T.D.N. intake. This figure was used as a measure of net efficiency for milk production. The formulas used in these calculations and the method of calculating efficiency on the caloric basis also are indicated in table 2.

The gross efficiency calculated by excluding only weight changes is probably

the most practical figure for comparing efficiency of milk production. Control cows consumed about 60 lb. (range 58–62) of T.D.N. (excluding weight gain) to produce 100 lb. of 4 per cent F.C.M. The thyroprotein-fed cows consumed

TABLE 2

Efficiency of milk production from the 50th to 305th day of lactation

Cow no.	F.C.M. produced	T.D.N. consumed	Weight gain	T.D.N. for maintenance[a]	T.D.N. per 100 lb. F.C.M.		
	50th to 305th day of lactation				Total[b]	Corrected for weight gain[c]	Corrected for gain & maintenance[d]
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
				Control cows—group 1.			
H-263	7,650	4,745	85	2,311	62.0	58.1	27.9
J-485	5,472	3,640	68	1,678	66.6	62.1	31.5
J-491	5,036	3,134	50	1,539	62.2	58.7	28.2
Av.	6,053	3,840	68	1,843	63.6	59.6	29.2
				Caloric efficiency[e]—	29.4%	31.4%	64.0%
				Cows fed thyroprotein and T.D.N. at 100%—group 2-A.			
H-268	5,380	4,092	11	1,930	76.1	75.3	39.5
H-269	3,013	3,008	80	1,711	99.9	90.5	33.7
J-487	6,014	3,555	21	1,461	59.1	57.9	33.6
Av.	4,802	3,552	37	1,701	78.4	74.6	35.6
				Caloric efficiency[e]—	23.8%	25.1%	53.0%
				Group 2-B. Cows fed thyroprotein and T.D.N. at 125% during the last 120 days.			
H-262	9,536	5,895	59	2,178	61.8	59.6	36.8
J-488	5,123	3,609	0	1,460	70.4	70.4	41.8
J-489	6,106	4,352	72	1,593	71.3	67.1	41.0
J-490	6,690	3,802	21	1,475	56.8	55.7	33.7
Av.	6,864	4,415	38	1,676	65.1	63.2	38.3
				Caloric efficiency[e]—	28.7%	29.6%	48.9%
				Group 2-C. Cows fed thyroprotein and T.D.N. at 125% during this period.			
H-266	9,267	6,014	33	2,056	64.9	63.6	41.5
J-496	7,917	4,725	38	1,514	59.7	58.0	38.9
Av.	8,592	5,370	36	1,785	62.3	60.8	40.2
				Caloric efficiency[e]—	30.0%	30.8%	46.6%

[a] Obtained by summation of maintenance requirements calculated every ten days.

[b] $\frac{\text{Column } 3 \times 100}{\text{column } 2}$

[c] $\frac{(\text{Column } 3) - (3.53 \text{ column } 4) \times 100}{\text{column } 2}$

[d] $\frac{(\text{Column } 3) - (3.53 \text{ column } 4 + \text{column } 5) \times 100}{\text{column } 2}$

[e] Caloric efficiency $= \frac{340 \text{ F.C.M.} \times 100}{1814 \text{ T.D.N.}} = \frac{1870}{\text{column 6, 7, or 8.}}$

about 65 lb. (range 56–90) of T.D.N. to produce 100 lb. of 4 per cent F.C.M. On a caloric basis, the control cows showed a gross efficiency of 31 per cent.

The average for all thyroprotein-fed cows was 28.1 per cent which was not significantly different from that of the control cows. Neither figure was significantly different from the value of 30 per cent as given by Brody (8). The results of this experiment indicate that feeding a cow thyroprotein does not change significantly gross efficiency for milk production.

Net efficiency for milk production has been shown to decrease as the level of feeding increases, but gross efficiency is essentially independent of level of feeding (8, 11). For this reason it is not valid to compare the values obtained for net efficiency of cows fed extra nutrients (groups 2-B and 2-C) with the cows fed at requirement (groups 1 and 2-A).

All cows in this experiment had values for net efficiency in agreement with the values reported for normal cows fed at their respective levels of T.D.N. intake (8). This indicates that the net efficiency for milk production based on

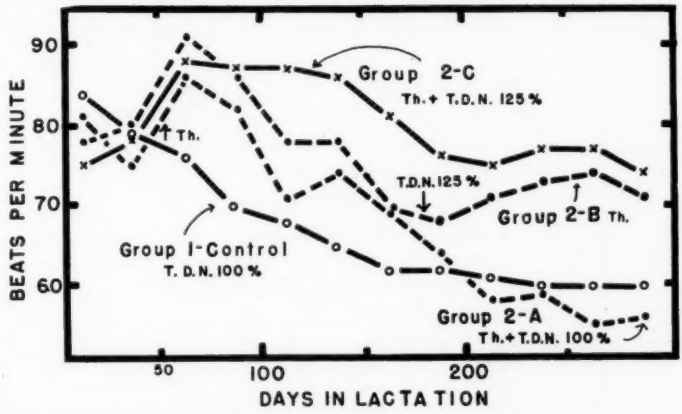

FIG. 3. Effects of feeding thyroprotein with different T.D.N. intakes on the average heart rates of the experimental animals.

the small number of cows fed thyroprotein in this experiment is essentially the same as that of normal cows fed at the same level of T.D.N. intake.

Effect on heart rate. The average heart rate of each group in relation to stage of lactation is presented in figure 3. Heart rates were taken with a stethoscope placed on the chest wall in the region of the heart. The rates were observed once every 5 to 10 days, but are presented in the graph as averages by 25-day intervals.

All cows had heart rates near 80 beats per minute during the first 50 days of lactation. Then the heart rate normally decreased to near 60 at mid-lactation and stayed near this level for the remainder of the lactation. The heart rate increased an average of about ten beats per minute during the first 25 days after thyroprotein was fed. The heart rate then decreased at an abnormally rapid rate in the cows that were fed thyroprotein and T.D.N. at requirement (groups 2-A and 2-B). However, the heart rate increase was sustained until

mid-lactation in the cows fed extra T.D.N. simultaneously with the thyroprotein and then decreased gradually. By mid-lactation the heart rate of cows fed thyroprotein and T.D.N. at requirement had decreased considerably and approached the value for normal cows. After mid-lactation the cows that remained on thyroprotein and T.D.N. at requirement (group 2-A) had heart rates that were similar to those of normal cows. However, when T.D.N. was fed at 125 to 150 per cent of requirement after mid-lactation (group 2-B) the heart rate was increased markedly and the increase persisted. Sykes *et al.* (21) and Ritzman and Benedict (18) have shown that heart rate was affected by level of T.D.N. intake.

Effect on butterfat percentage. The butterfat tests by 10-day periods for

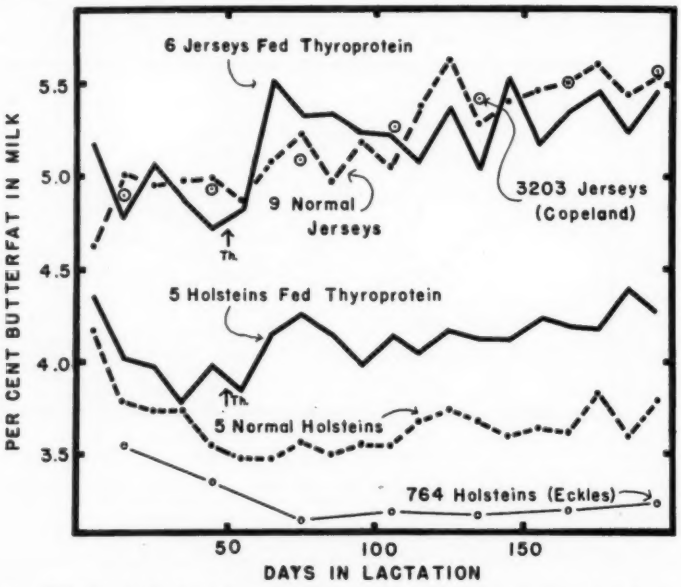

FIG. 4. Effects of feeding thyroprotein on the butterfat percentages and some values from the literature.

the control group and experimental groups for Jerseys and Holsteins, and also some values from the literature (23), are presented in figure 4. Since there were few control animals in the thyroprotein experiment, butterfat tests were obtained from other first-lactation heifers in the herd that had received the same ration. This provided a larger group of control cows in each breed for comparison with the cows fed thyroprotein. Data obtained by Copeland from monthly Jersey Register of Merit records as cited by Turner (23) were considered a reliable standard with which to compare our butterfat tests. The tendency toward an increase in butterfat percentage as lactation progresses is evident in Copeland's data and all our groups (fig. 4).

Butterfat percentages given in the literature for Holstein cows are much lower than the percentages for the experimental and some control Holstein cows in our herd. In past years the Holstein heifers in our herd have had lower butterfat percentages than the heifers in the herd at the present time. Hence the values for the standard and control Holstein group are inherently lower throughout the entire lactation than for the Holstein cows fed thyroprotein.

Soon after thyroprotein feeding was started, there was a marked increase in the mean fat percentage in the milk of the experimental Jersey and Holstein cows. Average butterfat tests during the month before thyroprotein took effect were compared with those during the month afterwards. As judged by the "t" test they showed a highly significant (1 per cent level) increase after the drug was fed in both the experimental Jersey and Holstein groups. No other month-to-month changes in butterfat tests were significant except the decrease from the first to the second month in both the control and experimental Holstein groups. Comparisons of mean butterfat tests for each monthly period between control and experimental Jersey groups showed no significant difference by the "t" test at any month of lactation. Comparing the mean monthly butterfat tests of the experimental Jerseys with those taken from the data of Copeland (23) for R. of M. Jerseys showed no difference except for a significant increase (5 per cent level) for the first month of thyroprotein feeding.

Comparing the mean monthly butterfat tests of experimental and control Holstein groups showed that the increase due to thyroprotein feeding was significant for only the first month of feeding. The results show that a significant increase in butterfat percentage was observed during the first 30 to 40 days of thyroprotein feeding. After about a month of thyroprotein feeding, butterfat tests of thyroprotein-fed cows were not significantly different from those of normally fed cows.

Rate of thyroprotein feeding. Our data do not indicate much difference in the amount of stimulated milk production caused by feeding thyroprotein at either 1.0 or 1.5 g. per 100 lb. body weight. The amount of response appears to be limited more by the individuality of the cow than by the difference in these two rates of thyroprotein feeding. It is possible that with a larger or more homogenous group of cows a better estimate of response in respect to dosage may be obtained.

Cow J-604, when fed thyroprotein at 0.6 g. per 100 lb., showed very little or no stimulation in milk production and in heart rate. However, cow H-272, fed thyroprotein at this same rate but with extra nutrients, showed some stimulation in milk production and in heart rate after the drug was fed. The difference in response of these two cows to the drug may have been due to individual variability of the cows and/or differences in feed intake.

It appears that feeding thyroprotein at the rate of 0.6 g. per 100 lb. is too low to elicit a marked response with some cows. Whether smaller amounts than 1.0 g. per 100 lb. can produce a desirable stimulation without the feeding of extra nutrients is still under investigation.

DISCUSSION

These experiments have verified the results that others have obtained in short term trials in showing that feeding thyroprotein stimulates milk production in most cows. In addition, individual cows evidently differ widely in their response to the drug, and cows that were poor producers have not been changed into good producers by feeding them thyroprotein. During the month after thyroprotein feeding was started, the F.C.M. production of the cows that were fed thyroprotein was 108 per cent of their pre-experimental level. The corresponding figure for the control cows was 91 per cent. This gives a difference of 17 per cent in the amount of milk produced during the first month of feeding thyroprotein. This increase is comparable to that which other investigators observed in short term trials as a result of feeding thyroprotein (3, 4, 15, 25).

Our experiments show that the period of stimulated milk production due to feeding thyroprotein can be lengthened by simultaneously increasing the feed intake of the cow to approximately 125 per cent of requirement. Also, the excessive decreases in body weight that occur when thyroprotein is fed and the T.D.N. intake is limited to requirement do not occur when the T.D.N. intake is increased to 125 per cent of requirement. The first report from this station (13) and also the report by Hibbs and Krauss (10) pointed out that milk-production and body-weight changes due to feeding thyroprotein were dependent upon the nutrient intake of the cow.

Our limited data on total milk production for the entire lactation do not answer the question of whether cows fed thyroprotein and T.D.N. at requirement will produce more than those not fed the drug. Indications are that total production for the lactation period would not be changed markedly as a result of feeding thyroprotein under these conditions. However, feeding T.D.N. at 125 per cent of requirement simultaneously with thyroprotein appears to have resulted in increased production for the first lactation. Whether these cows will continue to produce increased amounts of milk during subsequent lactations is under investigation. The amount of stimulation obtained by feeding control cows T.D.N. at 125 per cent of requirement was not investigated during the first lactation.

One cannot help noticing the similarity between the trends in milk production (fig. 1) and heart rate (fig. 3). In these experiments the responses observed in heart rate and in milk production are similar. The results indicate that heart rate and level of milk production are functions of stage of lactation, T.D.N. intake and thyroid stimulation.

Booth *et al.* (6) failed to find a relation between nutrient intake and heart rate, which is not in agreement with our data or the data of others (18, 21). However, the actual T.D.N. intake of the cows used by Booth *et al.* was not determined. Also, it has been shown that variations in heart rate may be produced by technics used in determining rate, stage of pregency, the drying-off process and other factors (2, 22). These facts could explain why Booth *et al.* (6) found no relation between heart rate and level of concentrate feeding.

Thyroprotein administration has been reported (1, 3, 4, 6, 10) to have incon-

sistent effects on the butterfat percentage of the milk. Some cows have shown increases, while others showed no change in butterfat percentage as a result of receiving thyroprotein. Others (15, 16, 19, 20, 25) have stated that thyroprotein or thyroxine administration increased the fat percentage of the milk. In this experiment about 50 per cent of the first-lactation animals showed a marked increase in butterfat percentage after thyroprotein was fed. No animal showed a decrease in butterfat percentage. Evidently feeding thyroprotein increases the butterfat percentage in the milk of some cows and not of others.

However, in comparing the butterfat tests of the cows that were fed thyroprotein with tests published in the literature and with tests of our limited control group, no indication was found that thyroprotein feeding had any prolonged effect on fat percentage. According to our data, after the first 30–40 days of thyroprotein feeding there are no marked differences in butterfat tests between the cows fed thyroprotein and control cows. It appears that the butterfat test of thyroprotein-fed animals is increased only temporarily over that of normal or control animals.

The gross efficiency for milk production of cows with and without thyroprotein administration was within the normal range and near the average value of 30 per cent as given by Brody (8). Brody further stated that the net efficiency for milk production was about 60 per cent when cows were fed T.D.N. at requirement. However, cows fed T.D.N. at higher levels have a lowered net efficiency for milk production. The values for net efficiency obtained with or without thyroprotein administration are similar to the values cited by Brody for corresponding T.D.N. intakes. Using our data to calculate the efficiency of milk production, the results indicate that feeding thyroprotein has not changed the over-all efficiency. Whether efficiency of the various metabolic processes has been altered cannot be determined from our data. It is interesting to note that workers (7, 24) have fed thyroprotein to pigs and found that efficiency of gain was reduced whenever thyroprotein was fed in amounts that would change the rate of gain. The decreased body weight gains and losses in body weight when steers and calves were fed thyroprotein (21, 22) and the data on pigs might indicate that the maintenance requirements of these animals were increased as a result of the thyroprotein feeding. One can only speculate whether feeding thyroprotein to the cow with the resulting increase in heart and respiration rates tends to change the maintenance requirement of the milking cow without altering the over-all efficiency for milk production.

From a practical viewpoint, efficiency is not altered by feeding thyroprotein at the rates used in this experiment. The cows in this experiment consumed about 60 lb. of T.D.N. to produce 100 lb. of 4 per cent F.C.M. whether or not thyroprotein was fed. The data indicate that normal input-output relationships were not disturbed, at least in the first lactation. However, the economy of feeding thyroprotein would depend on the relative cost of the increased nutrients and thyroprotein and the relation of these costs to the value of the increased milk production. More extensive experimental work on this phase of thyroprotein feeding is needed.

For the practical dairyman there are other important factors to consider. The effects that thyroprotein feeding might have on production in succeeding lactations and on the lifetime performance of the cow are undetermined. The effects on reproductive performance, resistance to disease and possible pre- or post-natal effects on the calf must be considered. These aspects will be considered in future reports from this station.

SUMMARY AND CONCLUSIONS

Thyroprotein has been fed to a group of 11 cows for the first lactation beginning 50 days post-partum and continuing an average of 300 days. The following observations have been made:

1. Extra feed must be fed in order to maintain body weight and milk production.

2. If extra feed is not fed, the cows show a period of stimulated milk production followed by a rapid decline in both body weight and milk production.

3. When extra feed (25 per cent above requirement) and thyroprotein were fed simultaneously, high production was maintained throughout the lactation period.

4. The gross efficiency for milk production apparently was unaltered by feeding thyroprotein. Also the net efficiency for the corresponding level of nutrient intake was unchanged.

5. The fat percentage in the milk of thyroprotein-fed cows was increased above that of normally-fed cows for a period of only 30 to 40 days.

6. Heart rate was increased by feeding thyroprotein. The increase was sustained throughout lactation when T.D.N. was fed at 125 per cent of requirement, but the heart rate was increased temporarily, then decreased rapidly if T.D.N. were fed at requirement. It was pointed out that heart rate, like milk production, definitely was related to level of T.D.N. intake, stage of lactation, and thyroprotein administration.

7. Cows that are poor producers respond less than cows that are good producers, so it is not possible to make a good cow from a poor cow by administering thyroprotein.

8. Since all the effects of feeding thyroprotein to producing dairy cows have not been determined at this time there is no sound basis on which one can make recommendations to the practical dairyman concerning the advisability of feeding thyroprotein.

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SEMEN PRODUCTION AND FERTILITY OF DAIRY BULLS FED RATIONS CONTAINING PROTEINS OF PLANT AND ANIMAL ORIGIN

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The effect of the source of protein in the rations of mature dairy bulls used in artificial breeding has received little attention in this country (14). However, a number of Russian workers (16, 19, 20, 28, 29) have stressed the necessity of including animal proteins in the rations of stud bulls.

The feeding and management practices in Russia differ considerably from those in general use in this country. Bulls used in studs in Russia in many instances have been fed rations very low in protein (19, 28, 29). Also, their practice (12, 13, 16, 20, 33) has been to use bulls periodically and collect semen from them much more frequently during these periods than has been done in the United States. Furthermore, it appears that only a few bulls have been studied and these for short periods of time by these workers (12, 13, 20, 28). Since the authors have seen only the abstracts of these papers, the experimental designs employed cannot be evaluated.

Although the Russian workers have concluded that the animal proteins contained in blood meal and skim milk caused marked improvement in sperm resistance and concentration and apparently affected spermatogenesis favorably, the authors believe these conclusions are open to question. Bratton (7) found that for dairy cows, protein quality apparently was of little practical importance when the ration consisted of common roughages and concentrates. Tankage, fish meals and dried blood meal have been compared with vegetable protein supplements such as soybean oil meal, cottonseed meal and linseed meal in the ration of lactating dairy cows (2, 3, 4, 17). Milk production was either slightly lower or about equal for the cows fed the animal proteins as compared to the production of the cows fed the protein supplements of vegetable origin. Recent studies (8, 10, 11, 21, 32, 34, 35, 36) have shown that proteins may be synthesized by the bacteria of the rumen from simple nitrogenous compounds such as urea and some ammonium compounds. Such evidence suggests there should be no advantage in feeding animal proteins as compared to vegetable proteins as supplements in the ration of dairy bulls when the level of digestible protein is adequate.

Because the evidence published by the Russian investigators (16, 19, 20, 28, 29) was not conclusive and seemed to be contradicted by the investigations on quality of protein for the dairy cow and experiments involving the synthesis of proteins in the rumen, the experiment reported herein was undertaken. The

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purpose of this investigation was to relate differences in measurable semen characteristics and relative fertility of dairy bulls used routinely in artificial insemination to concentrate mixtures containing primarily either animal or vegetable sources of supplementary protein.

EXPERIMENTAL METHODS AND MATERIALS

Feeds used. Since cereal grains and their by-products are decidedly inferior to such food as milk in the quality of protein contained and since the proteins of corn grain are low in lysine and tryptophane (18), an all-cereal concentrate mixture containing corn gluten feed as the protein supplement was chosen for study. In contrast, skim milk powder, which contains an abundance of protein of exceptionally high quality and which supplements so as to correct the deficiencies in the proteins of the cereal grains, was chosen as an animal source of supplementary protein. Soybean oil meal, though of plant origin, is especially rich in protein, and its protein is of excellent quality (18). Therefore, it was chosen as representative of a good plant protein. Timothy hay was used as the only roughage because it is low in protein, is readily available and is not recommended by Morrison (18) when a feed such as corn gluten feed, corn gluten meal, brewers' dried grains or distillers' dried grains is the protein supplement.

In a previous study reported by the authors (6), it was found that a concentrate mixture containing approximately 12 per cent protein, when fed with mixed hay, supplies sufficient protein for the maintenance of body weights as well as semen production of bulls. However, since timothy hay was selected instead of mixed or legume hay and since comparisons between protein supplements were being made, the concentrate mixtures were made to contain approximately 16 per cent protein (calculated). Also, it was reported by these authors (6) that T. D. N. intakes of approximately 110 per cent of the recommended maintenance requirements for dry dairy cows of equivalent weights (18) were required to maintain the body weights of bulls. The amounts of roughage and concentrates were adjusted to furnish this level of T. D. N. intake, with approximately 40 per cent of the T. D. N. intake furnished by the concentrates.

The ingredients used to make up each of the concentrate mixtures, the chemical analyses of each mixture and that of the hay, and the cost per ton of each concentrate are given in table 1.

The feeding schedule for each bull was based on initial body weight. This schedule was not altered as a consequence of subsequent changes in body weight of individual bulls during the three experimental periods.

Management of bulls. All bulls were exercised daily in a small dry lot or on a mechanical exerciser. The latter exercise was equivalent to a slow walk for approximately 1 mile. Throughout the experiment, each bull was used for service approximately once every 13 days, at which time one, two or three ejaculates were collected. Occasionally a fourth ejaculate was collected.

The experimental design. For the study of the three protein supplements, a "double change over" design, as described by Cochran *et al.* (9), involving

three 120-day periods, was employed. One Ayrshire, eleven Holstein-Friesian, and six Guernsey bulls, averaging 7.64 ± 0.44[3] years of age and 1866 ± 67[3] lb. in body weight, were selected from the stud of the New York Artificial Breeders' Cooperative, Inc., on the basis of their previous semen production and fertility records and the likelihood that they would remain in active service throughout the 360-day experiment. Assignment to the six groups was made on the basis of information available concerning each bull's semen production, fertility and body weight. The assignment of the sequences of the concentrate mixtures A, B and C to each bull was done in the manner described by Cochran *et al.* (9).

TABLE 1

Ingredients and chemical composition of the concentrate mixtures and hay

	Concentrate mixtures			Hay timothy
	A	B	C	
Ingredients	(*lb.*)	(*lb.*)	(*lb.*)	
Hominy feed	635	760	820	
Ground oats	500	610	660	
Wheat bran	200	200	200	
Soybean oil meal	.....	.....	280	
Skim milk powder	.....	390	.....	
Corn gluten feed	625	.....	.....	
Steamed bone meal	20	20	20	
Salt	20	20	20	
Total	2000	2000	2000	
Chemical composition[a]	(%)	(%)	(%)	(%)
Moisture	10.49	10.16	14.46	7.76
Crude protein	14.00	14.78	14.73	6.03
Ether extract	3.69	3.12	2.08	1.46
Crude fiber	8.87	6.12	8.17	39.23
Nitrogen-free extract	57.15	60.06	55.35	41.77
Ash	5.80	5.76	5.21	3.75
Total	100.00	100.00	100.00	100.00
Total digestible nutrients (%)[b]	76.13	77.60	77.05	51.60
Cost per ton (dollars)[c]	70.60	120.50	71.20	

[a] By analysis.
[b] By calculation.
[c] Actual cost at time of experiment.

The experimental design employed randomly distributed the seasons of the year throughout the treatments.

Measurements of the results. The following criteria were used to evaluate the semen samples: volume, per cent of motile spermatozoa (5), concentration (numbers of spermatozoa per mm.[3]) (25), and methylene blue reduction time (37). Each semen sample which met the specifications for use in artificial insemination was diluted with yolk-citrate-sulfanilamide diluent (27) and shipped by the Cooperative to its affiliated local units throughout New York State for

[3] Standard error of mean.

use in inseminating cows. Semen samples which did not meet the specifications for shipment were discarded. This fact itself was used as an additional over-all criterion of semen quality.

The semen dilution rates employed varied from 1:30 to 1:125, depending on the need for semen on any particular day. These varying rates were not confounding factors within the experiment, as has been shown by investigations of Salisbury (24, 26).

The fertility of the semen samples was determined from the number of services to cows being bred artificially for the first time or for the first time after calving and whether or not these cows returned to artificial service during a period of 60 to 90 days. Fertility is expressed as the per cent non-returns to first services.

In order to establish the initial body weight and an indication of body weight changes of each bull, the bulls were weighed on 3 consecutive days at the beginning of the experiment and at the end of each 120-day experimental period.

Data were recorded routinely on the characteristics of each ejaculate of semen produced by each bull at each collection period, on the fertility of the semen used for insemination and on the changes in body weights of the bulls. For the statistical analyses of the semen characteristics, the values for each ejaculate were used as single observations. Since the number of ejaculates per bull varied, the significance of treatment differences was tested after correcting for unequal subclass numbers in the analysis of variance (15, 31).

RESULTS AND DISCUSSION

The results of the investigation are presented in three parts, namely, the characteristics of the semen produced, the fertility of the semen used and the changes in body weights of the bulls.

Characteristics of the semen. The number of ejaculates and the mean values for the different characteristics used in the evaluation of the semen are shown in table 2. These means represent first, second, third and, in a few instances, fourth ejaculates.

The analyses of variance of the data on the semen characteristics revealed that except for significant differences between individual bulls, groups of bulls and experimental periods, all of which were expected, the only significant difference for treatments was between the average volume per ejaculate produced by the bulls on concentrate mixtures *A* or *B* as compared to those on concentrate mixture *C*. Although the average volume of the ejaculates of the bulls on concentrate mixture *C* was significantly lower (at the 1 per cent level of probability) than that of the bulls fed either mixture *A* or *B*, this was not considered to be a difference of any practical importance. The results as shown in table 2 are interpreted to mean that corn gluten feed, skim milk powder or soybean oil meal as protein supplements in the concentrate mixture fed with timothy hay to bulls used routinely in artificial insemination are of equal value for semen production.

TABLE 2

Summary of semen characteristics

Concentrate mixture	Semen characteristics				
	Vol.	Motile sperm	Number of sperm per mm.³ semen	Methylene blue reduction time	Ejaculates shipped
	(*ml.*)	(%)	(×10^3)	(*min.*)	(%)
A—Corn gluten feed					
Mean	6.33	72.8	1160	5.75	68.0
Number of ejaculates	253	253	253	229	253
B—Skim milk powder					
Mean	6.39	69.5	1159	5.48	65.2
Number of ejaculates	260	256	258	226	259
C—Soybean oil meal					
Mean	5.85	69.4	1133	5.71	61.3
Number of ejaculates	279	275	277	239	279

These findings are in agreement with those of Reid *et al.* (22, 23) who compared the effect of feeding a simple concentrate mixture containing ground yellow corn, beet pulp, corn gluten meal, cane molasses and iodized salt with that of feeding a complex mixture containing linseed meal and soybean meal as the protein supplements on the semen production of young bulls. These workers (23) report that "regardless of the diet fed, the concentration of spermatozoa in semen, the initial motility, the degree of livability, the size of the spermatozoa, the quantity of total reducing substances, reducing substances in oxidized state, potential reducing capacity, ascorbic acid, and the initial pH of the semen of bulls" from 18 to 33 months of age were similar. However, it should be pointed out that these investigators (22, 23) fed no protein supplement of animal origin, and they were not dealing with mature dairy bulls.

On the other hand, the results reported in this paper do not confirm those of the Russian investigators (16, 19, 20, 28, 29).

Fertility of the semen used. Fertility data were obtained only on those ejaculates meeting the relatively high standards of semen quality required by the New York Artificial Breeders' Cooperative, Inc. These data, presented in summary form in table 3, show, by treatments, the total number of first serv-

TABLE 3

Summary of fertility data

Treatments	Fertility criteria		
	Total first services	Total 60–90-day non-returns	% 60–90-day non-returns
Concentrate mixture:			
A—Corn gluten feed	15,827	10,058	63.5
B—Skim milk powder	16,692	10,285	61.6
C—Soybean oil meal	15,252	10,026	65.7

ices, the total number of 60–90-day non-returns and the calculated per cent non-returns. When the per cent non-returns for each semen sample was used as a single observation, the analyses of the variance showed that the semen used from bulls fed concentrate mixture *C* (soybean oil meal as the protein supplement) gave an average fertility level that was significantly higher at the 1 per cent level of probability than that from the bulls fed concentrate mixture *B* (skim milk powder as the protein supplement). However, the average difference in fertility of the semen from bulls on all three of the concentrate mixtures was small.

The authors have no explanation as to why the fertility of the semen from bulls receiving the concentrate mixture containing skim milk powder as the

TABLE 4

Initial body weights and body weight changes

Bull	Initial body weight	Body weight changes (Initial minus end of period)[a]		
		End of period 1	End of period 2	End of period 3
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
1	1898	62	82	−55
2	1975	65	57	−82
3	1525	29	101	−38
4	2094	− 5	74	−25
5	1959	56	139	−17
6	1945	−22	37	.....
7	2044	96	146	−12
8	1959	−23	86	1
9	2013	90	39	−28
10	2123	5	19	−89
11	2207	63	109	−97
12	2441	12	101	.....
13	1421	64	19	−19
14	1623	47	79	−16
15	1559	−27	19	−47
16	1695	5	99	18
17	1642	8	113	−52
18	1458	− 4	84	− 5
Means	1866	29	78	−35

[a] Body weight changes were based on the weight at the beginning of the experiment.

protein supplement was the lowest. Certainly, there appears to be no advantage of feeding skim milk powder as a protein supplement instead of corn gluten feed or soybean oil meal for periods of 120 days to bulls used for artificial breeding when these bulls are receiving timothy hay as the only roughage. Since the cost of the concentrate mixture containing the skim milk powder is almost twice that of either of the other concentrate mixtures (table 1), these findings are of economic importance.

Body weight changes. The initial body weight and body weight changes for individual bulls, and the respective means for all bulls used in this experiment are given in table 4.

As stated previously, an attempt was made in this study to maintain body weights of the bulls by feeding at the rate of 110 per cent of the recommended

T. D. N. requirements for the maintenance of dry dairy cows. As shown in table 4, the body weight changes during periods 1 and 2 were mostly increases, but during period 3, mostly decreases. The reason for the decrease in body weight of practically all the bulls during the last experimental period is not apparent, since the composition of the concentrate mixtures and the quality of the timothy hay fed appeared to be about the same through the experiment. However, the last period was during the warm months, June, July, August, September and October.

SUMMARY

Using 18 bulls, 11 Holstein-Friesians, 6 Guernseys and 1 Ayrshire, studies were conducted relating differences in measurable semen characteristics and relative fertility to concentrate mixtures containing corn gluten feed, skim milk powder or soybean oil meal as the protein supplement when fed with timothy hay as the only roughage.

The results, as judged by the averages for volume of semen per ejaculate, per cent of motile spermatozoa and their rates of motility, the number of spermatozoa per mm.^{3} of semen, the methylene blue reduction time and the per cent of usable samples during a 120-day period, showed that corn gluten feed, skim milk powder and soybean oil meal were approximately equal in value as protein supplements in the concentrate mixture.

Based on 60 to 90 days non-returns to first service cows, the average fertility levels of the semen produced when the bulls were fed corn gluten feed, skim milk powder and soybean oil meal were 63.5, 61.6 and 65.7 per cent, respectively. While the average per cent non-returns was significantly higher during the periods when soybean oil meal was fed as compared to the periods when skim milk powder was fed, the difference was relatively small and it is doubtful whether any real advantage lies with the soybean oil meal. In these studies, animal protein was not superior to the plant proteins. The monetary economy of the particular plant protein sources used in these trials was much greater than for the animal protein source.

A T. D. N. intake of 110 per cent of Morrison's recommended requirements for the maintenance of dry dairy cows of equivalent weight resulted in consistent body weight increases for all bulls during the first 240 days but consistent decreases during the last 120 days of the experiment. While the decreases occurred during the summer and early fall months, it was not definitely established whether the observed decreases represented a true cause and effect relationship.

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A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDROGUAIARETIC ACID IN DAIRY PRODUCTS. III. ITS ANTIOXYGENIC PROPERTIES IN SWEETENED FROZEN CREAM

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Sucrose sometimes is added to cream which is to be stored frozen in order to lessen the undesirable destabilizing effect on the fat emulsion during freezing and thawing (3, 4, 6, 8, 9, 10, 13). Furthermore, some investigators (7, 9, 12) have found that added sucrose will retard slightly the development of oxidized flavor.

It has been shown (11) that nordihydroguaiaretic acid (NDGA) will retard the development of oxidized flavor in unsweetened frozen cream. The work reported herein consists of a study in which NDGA was used to retard the development of oxidized flavor during the storage of sweetened frozen cream.

EXPERIMENTAL PROCEDURE

Cream of both high and low quality was used in this study. Standard plate count (1), acidity and pH were the quality criteria (table 1).

TABLE 1

The standard plate count, titratable acidity and pH of the raw and pasteurized cream

	Low quality		High quality	
Raw Cream	*Series A & B*	*Series E & F*	*Series C & D*	*Series G & H*
Standard plate count/ml.	1,000,000	600,000	135,000	180,000
Titratable acidity (as % lactic acid)	0.19	0.18	0.125	0.13
pH (25° C.)	6.70	6.70	6.90	6.80
Pasteurized Cream	*150° F.*	*170° F.*	*150° F.*	*170° F.*
Standard plate count/ml.	5,500	500	300	400
Titratable acidity (as % lactic acid)	0.19	0.17	0.13	0.13
pH (25° C.)	6.65	6.70	6.75	6.90

The different batches of cream were standardized to contain 40 per cent milk fat and 10 per cent sucrose. NDGA was added before pasteurization as a 15 per cent solution in propylene glycol or as a 5 per cent water suspension. The concentrations of NDGA were computed on the basis of the fat content of the cream. When used, copper was added before pasteurization at a concentration of 0.5 p.p.m. as a 0.5 per cent aqueous solution of copper sulfate.

The cream was pasteurized in well-tinned equipment, cooled to 40 to 45° F., sealed in tinned cans holding 300 ml. and stored at −12 to −20° F. To examine for monthly flavor defects the frozen cream was thawed by holding it 24 hours at 40° F. It was then held at 40° F. for 1 week and again examined for flavor defects. The judging panel consisted of three or more persons.

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RESULTS

The data in table 2 are typical of results obtained with cream placed in storage during January and February. There were no significant differences in the antioxygenic effectiveness of the NDGA when added in propylene glycol solution and when added in a water suspension. Therefore, the data presented in table 2 include only results from cream treated with NDGA in propylene glycol solution.

The effect of concentration of NDGA. A concentration of 0.005 per cent of the antioxidant was more effective than one of 0.00125 per cent. This is shown in the results obtained with the cream of series D (table 2) at the end of 12 months and the cream of series B after storage for 8, 10 and 12 months. In the former case, oxidized flavor was present at the end of storage for 12 months at sub-zero temperatures plus 1 week at 40° F. in the cream containing 0.00125 per cent NDGA, but was not present in the cream which contained 0.005 per cent NDGA. In the latter case, oxidized flavor was present at the end of storage for 8 months at sub-zero temperatures in the cream containing 0.00125 per cent NDGA but was not detected in the cream containing 0.005 per cent antioxidant until the end of 12 months; at that time the intensity of the off flavor was less in the sample containing 0.005 per cent NDGA.

The effect of pasteurization temperature. Oxidized flavor development was retarded by pasteurization at 170° F. for 15 minutes. This substantiates the results obtained in other investigations dealing with the factors affecting the keeping quality of frozen cream (2, 5, 11).

Oxidized flavor was present after 2 months in the control sample of series B while it did not develop during storage at sub-zero temperatures until after 10 months in the similar cream (series F) which was pasteurized at 170° F. for 15 minutes. There was no oxidized flavor in the cream of series G, while the off-flavor was present at the end of 8 months storage in the control sample of the cream (series C) pasteurized at 150° F. for 30 minutes. The oxidized flavor was present after storage for 2 months in the control sample of series D while there was no development of typical oxidized flavor in the control sample of the similar cream (series H) pasteurized at 170° F. for 15 minutes.

While keeping quality of the control samples of the cream pasteurized at 170° F. for 15 minutes was superior to those pasteurized at 150° F. for 30 minutes, the former had a cooked flavor which persisted throughout the storage period. The keeping quality of the cream which was pasteurized at 150° F. for 30 minutes and which contained NDGA was comparable to that of the cream pasteurized at 170° F. for 15 minutes but which contained no added antioxidant.

The effect of quality of the cream. Oxidized flavor was present at the end of 8 months storage in the control sample of the high-quality cream of series C which contained no added copper, whereas there was no oxidized flavor development in the similar low-quality cream (series A). There was a similar difference in the keeping quality of the low- and high-quality cream containing added copper (series B and D, respectively).

TABLE 2

The antioxidant effect of NDGA added to sweetened cream stored at sub-zero temperatures

Treatment	Flavor criticisms											
	2 mo.		4 mo.		6 mo.		8 mo.		10 mo.		12 mo.	
	(1)[a]	(2)[b]	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Pasteurized at 150° F. for 30 min.												
Series A. Low quality cream. No copper added. No oxidized flavor in control, control + 0.00125% NDGA and control + 0.005% NDGA												
Series B. Low quality cream. 0.5 p.p.m. copper added.												
Control	1[c]	1	1	3	1	3	2	3	2	3	3	5
Control + 0.00125% NDGA	–[d]	–	–	–	–	–	–	–	–	–	–	1
Control + 0.005% NDGA	–	–	–	–	–	–	–	–	–	–	–	–
Series C. High quality cream. No copper added.												
Control	–	1	–	2	±[e]	3	1	4	3	4	3	4
Control + 0.00125% NDGA	–	–	–	–	–	–	–	–	–	–	–	–
Control + 0.005% NDGA	–	–	–	–	–	–	–	–	–	–	–	–
Series D. High quality cream. 0.5 p.p.m. copper added.												
Control	1	2	2	3	3	4	4	5	4	5	4	5
Control + 0.00125% NDGA	–	–	–	–	±	±	1	1	1	1	2	2
Control + 0.005% NDGA	–	–	–	–	–	–	–	–	–	–	1	1
Pasteurized at 170° F. for 15 min.												
Series E. Low quality cream. No copper added. No oxidized flavor in control, control + 0.00125% NDGA and control + 0.005% NDGA												
Series F. Low quality cream. 0.5 p.p.m. copper added.												
Control	–	–	±	1	±	1	±	1	1	2	2	2
Control + 0.00125% NDGA	–	–	–	–	–	–	–	–	–	–	–	–
Control + 0.005% NDGA	–	–	–	–	–	–	–	–	–	–	–	–
Series G. Low quality cream. No copper added. No oxidized flavor in control, control + 0.00125% NDGA and control + 0.005% NDGA												
Series H. High quality cream. 0.5 p.p.m. copper added.												
Control	–	±	–	±	–	±	±	±	±	±	±	±
Control + 0.00125% NDGA	–	–	–	–	–	–	–	–	–	–	–	–
Control + 0.005% NDGA	–	–	–	–	–	–	–	–	–	–	–	–

[a] Flavor judged immediately after taking cream out of storage and thawing it.
[b] Flavor judged after thawing cream and then holding it at 40° F. for 1 week.
[c] The numbers 1 to 5 indicate increasing intensities of the oxidized flavor defect.
[d] No oxidized flavor present.
[e] Flavor slightly 'off'. Not typically oxidized.

In the case of cream pasteurized at 170° F. for 15 minutes, there was little difference in the keeping quality of the high- and low-quality cream. The control sample of the low quality cream (series F) developed oxidized flavor at the end of storage for 10 months but the similar high quality cream (series H) did not develop the typical oxidized flavor during 12 months storage.

In the study, therefore, the low quality cream which was pasteurized at 150° F. for 30 minutes had a better keeping quality than the high quality cream pasteurized in the same manner. In the case of cream pasteurized at 170° F. for 15 minutes, the high quality cream had better keeping quality than the low quality cream pasteurized in the same manner.

The effect of holding the thawed cream at 40° F. for 1 week. Oxidized flavor sometimes developed in the cream which was held at 40° F. for 1 week although it did not have that off-flavor when it first was taken out of storage at sub-zero temperatures. This relationship was illustrated in the sample containing 0.00125 per cent NDGA in series B at the end of 12 months, in the control sample of series C at the end of 2, 4 and 6 months, and in the control sample of series F at the end of 4, 6 and 8 months.

After storage for 1 week at 40° F., oxidized flavor usually increased in intensity in the control samples which had that off-flavor when they first were taken out of storage. This is the case in the control samples of series B at the end of 4, 6, 8, 10 and 12 months, series C at the end of 8, 10 and 12 months, series D at the end of 2 months and Series F at the end of 10 and 12 months. However, the intensity of oxidized flavor did not increase during storage at 40° F. in the samples which contained NDGA. This is shown in series D at the end of 8, 10 and 12 months in the cream containing 0.00125 per cent NDGA and at the end of 12 months in the cream containing 0.005 per cent NDGA.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.005 per cent (butterfat weight basis) nordihydroguaiaretic acid were found to retard the development of oxidized flavor in sweetened frozen cream during storage for 12 months.

2. In the absence of added copper, the keeping quality of the cream which contained nordihydroguaiaretic acid and was pasteurized at 150° F. for 30 minutes was comparable to that pasteurized at 170° F. for 15 minutes but to which the antioxidant had not been added.

3. In this study, the low quality cream pasteurized at 150° F. for 30 minutes had a better keeping quality than the high quality cream similarly pasteurized.

4. During storage for 1 week at 40° F., oxidized flavor developed frequently in the control samples which did not have the off-flavor when they first were taken out of storage at sub-zero temperatures. This development of oxidized flavor occurred only in one instance in the cream which contained nordihydroguaiaretic acid.

5. During storage for 1 week at 40° F., the intensity of oxidized flavor usually increased in the control samples which had the off-flavor when they

first were taken out of storage at sub-zero temperatures. This did not occur in the oxidized samples which contained nordihydroguaiaretic acid.

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THE RELATIONSHIP OF ASCORBIC ACID TO THE DEVELOPMENT OF OXIDIZED FLAVOR IN MARKET MILK

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The flavor known as oxidized and often called cappy, cardboard, oily or tallowy, is one of the most troublesome off flavors in milk, especially during the winter months. Although much work has been done on oxidized flavor during the past quarter century, neither the cause nor the remedy of the defect has been determined satisfactorily. Data recently published seem somewhat contradictory to previous work on oxidized flavor. This study was conducted to gain more knowledge of the causative factors involved in the development of the oxidized flavor of milk and to seek some practical control measures.

REVIEW OF LITERATURE

This review will include only the work which seems to have a direct bearing upon this particular study.

After reviewing past research on the development of oxidized flavor in milk, Brown and Thurston (1) concluded that copper, sunlight and the presence of oxygen promote the development of oxidized flavor. Milk known as "spontaneous" milk will develop an oxidized flavor without the aid of either copper or light. Ascorbic acid and other anti-oxidants were found to retard or inhibit the development of oxidized flavor when added to milk. Their review of some research indicates several other factors such as feed, temperature and season that may be involved in the production or inhibition of oxidized flavor under commercial conditions. A more recent review by Greenbank (6) summarizes the early work and emphasizes newer research and theories. The possible role of ascorbic acid in the development and inhibition of oxidized flavor is well presented in this review.

Beck *et al.* (2) found a marked relationship between color intensity or carotene content of milk and its susceptibility to oxidized flavor, the higher carotene milk being less susceptible. They were successful in preventing oxidized flavor in raw milk by feeding a concentrate containing as little as 206 mg. of carotene per head daily to cows that consistently had been producing milk with this off flavor.

Josephson *et al.* (7) found that under experimental conditions of exposing milk to sunlight, the loss of ascorbic acid was rapid, with only insignificant quantities remaining after 30 minutes. Even in the shade the loss of this vitamin was quite rapid. In their survey of route deliveries they found that 70 percent of all retail milk is removed from the doorstep within 5 minutes after delivery, or is protected from light. The loss of ascorbic acid was found to

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be considerable while the milk was in transit if not properly protected from direct light.

Chilson (3) found that by adding ascorbic acid to pasteurized milk processed under laboratory conditions the development of the oxidized flavor could be retarded for several days. He suggested that an enzyme was present in skim milk which catalyzed the oxidation of the fat, since the oxidized flavor did not develop in milk prepared from skim milk which had been heated to 170° F. for 30 minutes and then mixed with unheated cream. He also reported no appreciable loss of ascorbic acid when milk was heated to 200° F. and held for 30 minutes, but about 7 per cent loss occurred when milk was held at 143° F. for 30 minutes. It was assumed that the heating of milk to above 170° F. destroyed the oxidizing enzyme which was responsible for the destruction of ascorbic acid and the development of this off flavor.

Gjessing and Trout (4) found that milk pasteurized at 67° C. (167° F.) for 30 minutes exhibited marked stability of ascorbic acid with no development of oxidized flavor after storage for 3 days, even when 0.13 mg. of copper per l. had been added. They believed that the protection was due to the liberation of sulphides and other reducing agents at this high temperature.

Olson and Brown (9) have demonstrated that washed cream from susceptible milk does not develop an oxidized flavor when contaminated with copper and stored for 3 days. However, when washed cream from the same source, likewise contaminated with copper, was fortified with ascorbic acid, a strong oxidized flavor developed.

More recent work by Krukovsky and Guthrie (8) has shown that complete and rapid destruction of ascorbic acid by light or hydrogen peroxide will prevent the development of an oxidized flavor. These workers also have shown that ascorbic acid is an important link in a chain reaction promoting oxidation of milk fat.

Greenbank (5) explains the effect of either addition or destruction of ascorbic acid in relation to changes in the oxidation-reduction potential. The ability of a sample of milk to develop an oxidized flavor seems to be related closely to the Eh value and the poising action of the milk. However, some workers do not consider the O-R potential as a reliable index to oxidized flavor development (10).

METHODS

In preliminary studies prior to the organization of this experiment, six lots of milk collected during November and December were treated with 1.5 g. of ascorbic acid per 100 lb. of milk. These samples were stored for 5 days and examined for oxidized flavor after 0, 2, 3 and 5 days. The six control samples developed a distinct oxidized flavor by the fifth day or earlier, while no oxidized flavor was detectable in any samples treated with ascorbic acid. In these comparisons no chemical analyses were made to determine the amounts of ascorbic acid present or the rate of destruction during storage.

In the first part of this investigation the relationship between the destruc-

tion of ascorbic acid in milk and the incidence of oxidized flavor was studied. The two trials, each consisting of four split batches of milk, represented a comparison between milk fortified with ascorbic acid and non-fortified milk. The first trial was conducted in February and March when the cows were on winter feed and the second trial in April and May when the cows were on grass.

In the second part of this investigation the effect of subjecting the milk to various light exposures upon the ascorbic acid content and the incidence of oxidized flavors was studied. All milk used was fortified with ascorbic acid as described above. In three trials the ascorbic acid content was determined before and after exposure to light, and each sample was analyzed for ascorbic acid and tasted for oxidized flavor daily during the 5-day storage period.

In the third part of the investigation the incidence of oxidized flavor was compared when samples were treated by adding 30 mg. of ascorbic acid per l. of milk to inhibit development of the flavor, and when the ascorbic acid was destroyed by exposure of the milk to intense sunlight or by oxidation resulting from the addition of 0.1 ml. of 30 per cent hydrogen peroxide per l. of milk. The effect of sunlight upon flavor also was tested by subjecting to sunlight the milk in which the ascorbic acid had been destroyed through the addition of hydrogen peroxide. The exposure to direct sunlight was for 0.5 hour in each instance.

In the fourth part of this investigation a preliminary test was made, whereby samples of milk were subjected to conditions similar to those of commercial handling and retail delivery. In the first trial, milk fortified with ascorbic acid was stored 24 hours, then carried in a covered delivery truck in standard bottle cases for 3 hours (5 to 8 a.m.) and then exposed to daylight in the shade for 0.5 hour (8 to 8:30 a.m.). Ascorbic acid determinations were made after each successive treatment and the milk was examined for oxidized flavor after storage for 3, 5 and 7 days. In the second part of the trial three quarts of unfortified milk were taken from the bottle filler; one quart was placed in storage as a control, and two quarts were carried on the delivery truck from 9:30 a.m. to 12 noon. Then one bottle was placed in the shade for 1 hour while the other bottle was subjected to direct sunlight for 15 minutes.

The milk used for comparisons was pasteurized by the holder method. All equipment used was of stainless steel construction except the cooler which was tinned copper with no copper exposed. Samples were stored for from 0 to 7 days in a cabinet refrigerator at 35 to 40° F. and protected from light. The ascorbic acid content of the original milk and the milk at selected periods during storage was determined by the method of Woessner *et al.* (11). All samples were tested organoleptically by two experienced judges of milk flavors at the same period when analyzed for ascorbic acid content. The ascorbic acid was added to the milk in the vat after pasteurization previous to cooling, at the rate of 1.5 g. per 100 lb. of milk. Ascorbic acid powder, sufficient for 100 gallons of milk, was dissolved in a pint of water before being added to the milk. The milk used was from the Kansas State College herd and had been found susceptible to the development of oxidized flavor.

RESULTS

Both the control milk and the ascorbic acid fortified milk averaged slightly higher in ascorbic acid content when the cows were grass-fed than when cows were fed winter rations (table 1). However, the addition of 1.5 g. of ascorbic acid per 100 lb. of milk increased the ascorbic acid content of the fortified milk to approximately three times that of the control milk in both trials. The average losses of ascorbic acid in the two trials with control milk were quite similar; they were in excess of one-fifth of the original content after one day's storage, about one-half by the second day, three-fourths by the third day, and more than 90 per cent after 5 days' storage. Although more milligrams of ascorbic acid were lost daily in the fortified milk than in the non-fortified, in general, the percentage loss was at a much less rapid rate. The average losses with fortified milk were quite similar in both trials; the loss during the first day of storage was less than one-tenth in both trials, slightly more than one-fifth for both trials after 2 days, about one-third by the third day, and about one-half of the original after 5 days of storage. Even after 5 days of storage, the fortified milk contained about one-half more ascorbic acid than the control milk contained originally. No oxidized flavors were detected in the fortified milk during a 5-day storage period in the first trial or during a 7-day storage in the second trial. However, in the control samples, oxidized flavors began to develop on the second day of storage and became increasingly frequent with longer storage periods. These results indicate that the addition of 1.5 g. of ascorbic acid to 100 lb. of milk was sufficient to inhibit the development of oxidized flavor during a normal storage period. The general effect of grass feeding was to delay the appearance of oxidized flavor by 1 additional day. This could be of considerable importance in commercial milk distribution.

Results of three trials to determine the effects of various periods of exposure and various intensities of light upon the ascorbic acid content of fortified milk are shown in table 2. In trial I, exposure of milk for 1 hour in the shade caused an immediate reduction from 38.3 to 23.2 mg. (about 40 per cent) in the ascorbic acid content, while exposure for the same period to direct sunlight caused a reduction from 38.3 to 8.6 mg. (about 78 per cent). Further reductions in ascorbic acid occurred throughout the period of storage. Oxidized flavors were detected on the third day of storage in milk that had been exposed to daylight in shade, but milk exposed to direct sunlight had a pronounced oxidized flavor after only 1 day of storage.

In trial II exposure of milk to daylight on a cloudy day for one-half hour caused a loss from 43.6 to 27.9 mg. (about 36 per cent) of the ascorbic acid present, and oxidized flavor developed after only 1 day of storage. The instability of ascorbic acid when milk is exposed to direct sunlight was measured in more detail during trial III. Milk exposed for 5 minutes lost approximately half of its ascorbic acid, and milk exposed 20 minutes lost about 90 per cent, while milk exposed for 40 minutes lost practically all ascorbic acid. The oxidized flavor was detected after storage for 1 day of milk which had been ex-

TABLE 1

The relationship of ascorbic acid destruction and the development of oxidized flavor in pasteurized milk

Trial	Number of Samples	Treatment	Days Stored						Days Stored				
			0	1	2	3	5	7	1	2	3	5	7
			av. mg. ascorbic acid/l. milk and % loss						*frequency of occurrence and intensity of oxidized flavor*				
I Feb. March (Winter ration)	4	None	14.7	11.6 21%	7.4 50%	3.4 77%	1.3 91%		4–[a b]	2+[c] 2–	2++ 2+++	1+++ 3++++	
	4	1.5 g. ascorbic acid added/ 100 lb. milk	48.6	44.0 9%	37.5 23%	33.2 32%	24.3 50%		4–	4–	4–	4–	
II April May (Grass-fed)	4	None	17.2	12.8 26%	8.1 53%	4.4 74%	1.1 94%	1.1 94%	4–	4–	1+ 3–	1+ 3++	2++ 2+++
	4	1.5 g. ascorbic acid added/ 100 lb. milk	51.2	48.6 5%	40.6 21%	34.3 33%	26.4 48%	18.6 64%	4–	4–	4–	4–	4–

[a] Figure indicates number of samples in each classification.
[b] – indicates no oxidized flavor.
[c] +, ++, +++, ++++ indicate increasing intensity of oxidized flavor.

TABLE 2

The relationship of time milk is exposed to light, and the ascorbic acid content and the development of oxidized flavor

Trial	Sample and treatment[a]	Ascorbic acid content	Days Stored				
			1	2	3	4	5
I[b]	Not exposed[c]	38.3	*mg. of ascorbic acid/l. milk and oxidized flavor value*				
	Exposed 1 hr. to daylight in shade	23.2	18.7 –[e]	18.3 –	 ++[f]	8.3 ++++	
	Exposed 1 hr. to direct sunlight	8.6	6.4 ++++	6.0 ++++	 ++++	1.1 ++++	
II[b]	Not exposed	43.6	37.7 –	32.0 –			
	Exposed 0.5 hr. to daylight	27.9	23.1 ++	18.5 +++			
III[b d]	Not exposed	43.7	–	–	–	–	–
	Exposed 5 min. to direct sunlight	22.6	+	+++	+++	+++	+++
	" 10 min. " " "	12.2	++	++++	++++	++++	++++
	" 20 min. " " "	4.6	++	+++	++++	++++	++++
	" 40 min. " " "	1.2	–	–	–	–	–
	" 60 min. " " "	1.2	–	–	–	–	–

[a] All milk used in these trials was fortified with approximately 1.5 g. of ascorbic acid per 100 lb. of milk.

[b] Trial I, clear day (9–10 a.m., Mar. 8, 1948); trial II, cloudy day (9: 30–10 a.m., Mar. 10, 1948); trial III, clear day (10: 50–11: 50 a.m., Mar. 19, 1948).

[c] Stored samples not checked for flavor.

[d] Ascorbic acid content of stored samples not determined.

[e] – indicates no oxidized flavor.

[f] +, ++, +++, ++++ indicate increasing intensity of oxidized flavor.

posed 5, 10 or 20 minutes. On succeeding days of storage the oxidized flavor became more pronounced. No oxidized flavor was detected in any of the stored samples of milk which had been exposed 40 or 60 minutes to direct sunlight. It would seem that the rapid and rather complete reduction in the ascorbic acid content of the milk tended to inhibit the development of the oxidized flavor. These latter samples had a pronounced cooked or burnt flavor after 24 hours of storage, but the off flavor gradually disappeared as storage time was extended.

Krukovsky and Guthrie (8) have reported that oxidized flavor in milk can be inhibited by the complete destruction of ascorbic acid through either exposure of the milk to direct sunlight or the addition of hydrogen peroxide to the milk to oxidize the ascorbic acid. A preliminary trial was conducted to compare ascorbic acid-fortified milk with milk having all the ascorbic acid removed by the use of either hydrogen peroxide or sunlight (table 3). The

TABLE 3

The relationship of ascorbic acid, hydrogen peroxide, and sunlight to the development of the oxidized flavor

Sample	Ascorbic acid/l. of milk	Days stored			
		1	2	3	5
		Oxidized flavor value			
	(*mg.*)				
1. Control milk		–[a]	–	+	++
2. No. 1 + 30 mg. ascorbic acid/l.		–	–	–	–
3. No. 1 + 0.1 ml. 30% H_2O_2/l.	1.1	–	–	–	–
4. No. 2 + 0.1 ml. 30% H_2O_2/l.	1.6	–	–	–	–
5. No. 1 exposed 1.5 hr. to direct sunlight	0.6	–	–	–	–
6. No. 1 exposed 0.5 hr. to direct sunlight		–	+	+	++
7. No. 2 exposed 0.5 hr. to direct sunlight		++++[b]	++++	++++	++++
8. No. 3 exposed 0.5 hr. to direct sunlight		–	–	–	–
9. No. 4 exposed 0.5 hr. to direct sunlight		–	–	–	–

[a] –indicates no oxidized flavor.
[b] +, ++, +++, ++++ indicate increasing intensity of oxidized flavor.

stored control sample developed an oxidized flavor in 3 to 5 days, indicating the susceptibility of the milk. The addition of ascorbic acid to the control milk inhibited the development of the off flavor during the 5-day observation period. The addition of 0.1 ml. of 30 per cent hydrogen peroxide to 1 l. of milk removed essentially all quantities of ascorbic acid from both the control milk and the fortified milk. Neither milk showed evidence of oxidized flavor.

Exposure of a sample of the milk to direct sunlight for 1.5 hours also removed essentially all ascorbic acid. No oxidized flavor developed in this milk during 5 days of storage. However, when a sample was exposed to direct sunlight for 0.5 hour, an oxidized flavor developed after 2 days of storage. Exposure of the fortified milk to direct sunlight for 0.5 hour caused the development of a pronounced oxidized flavor after 1 day in storage. When the samples to which hydrogen peroxide had been added were exposed to 0.5 hour of direct

sunlight, no oxidized flavor resulted during 5 days of storage. This indicates that ascorbic acid must be present before the sunlight can cause the development of the oxidized flavor. Other samples were treated with hydrogen peroxide and gave results similar to those shown in table 3.

The results of a study on milk subjected to commercial handling conditions were as follows: Fortified milk containing 46 mg. of ascorbic acid per l. lost about one-sixth of the ascorbic acid after 24 hours storage, only a slight additional amount was lost while on the truck and about one-third of the remaining ascorbic acid was lost when exposed 0.5 hour in the shade. Unfortified milk containing 13.2 mg. of ascorbic acid per l. showed no loss of ascorbic acid after 2.5 hours on the truck, but about one-half was lost when exposed for 1 hour in the shade; practically all the ascorbic acid was destroyed after 15 minutes of exposure to direct sunlight. No oxidized flavor developed in any of these samples, even after 7 days storage, showing that the milk at this time was much less susceptible to oxidation than the milk used in the previous experiments.

DISCUSSION

From a review of the literature and the results of these experiments, it appears that oxidized flavor in susceptible milk can be controlled either by the addition of ascorbic acid to the milk or by the complete and rapid oxidation of all ascorbic acid present in the milk. When ascorbic acid is added to milk it acts as a reducing agent which oxidizes more readily than the milk fat, therefore either preventing or prolonging the time required for fat oxidation and the development of an oxidized flavor (3). When the ascorbic acid is oxidized completely and rapidly, the development of oxidized flavor is prevented (8). Theories on the chemical reactions involved in this procedure are somewhat controversial. At present, the addition of ascorbic acid is the only method of commercial interest. It now has been established that the oxidized flavor can be controlled for several days by the addition of ascorbic acid if the milk supply is free from copper and is protected from sunlight. The price of ascorbic acid is low enough to justify its use as a flavor control measure when necessary. Ascorbic acid is tasteless in the milk and, being a natural food substance, any excess at the time of consumption will add to the food value of the milk. Ascorbic acid also is relatively easy to add, no expensive equipment being necessary. However, its use presents a number of problems when considsidered for commercial use, such as approval by regulatory authorities, proper handling of the milk in relation to sunlight and possible deceptive labeling to show increased vitamin C content of the milk. Due to the instability of ascorbic acid under many conditions, no guarantee should be made regarding the vitamin C content of milk at the time of either delivery or consumption. The protection of the milk from sunlight during the transportation period to the customer's refrigerator would be the greatest problem for many dairies. Present knowledge would indicate only wasted effort if ascorbic acid were added to milk not protected from sunlight at all times. Under certain conditions, a

more objectionable oxidized flavor might be developed in milk with added ascorbic acid than would be encountered in the original milk (table 3).

It has been established that oxidized flavor can be controlled by the rapid and complete removal of ascorbic acid by several methods (8). (The measurement of ascorbic acid may be used as a guide to the inhibition of flavor development. However, it is not established fully that the oxidation of ascorbic acid is the key factor to the development or inhibition of the oxidized flavor.) There are no commercial methods now available for the rapid destruction of ascorbic acid in large quantities of milk. Should machinery be developed which will remove the ascorbic acid economically and rapidly in the line of processing, this method may hold the greatest possibility for future control of oxidized flavors. The greatest disadvantage of the removal of ascorbic acid would be the difficulty in obtaining the approval of a process by the public and the medical profession. Willful removal of a natural vitamin from a universally-used food may be questioned. However, if a survey were made of all milk at the time of consumption, there is little doubt that most of it would have insignificant quantities of vitamin C present.

SUMMARY

The addition of ascorbic acid to milk at the rate of 1.5 g. to 100 lb. resulted in a three-fold increase in ascorbic acid content of the milk. Non-fortified milk lost more than one-fifth of its ascorbic acid during the first day of refrigerated storage in the dark, about half by the second day, three-fourths by the third day, and better than 90 per cent by the fifth day. Milk fortified with ascorbic acid lost less than one-tenth during the first day, slightly more than one-fifth the second day, about one-third by the third day, and about one-half after 5 days of storage. After 5 days the fortified samples contained about one-half more ascorbic acid than the control milk did originally. No oxidized flavors developed in the fortified milk in 5- or 7-day storage periods, while the control samples began to develop oxidized flavors after 2 days of storage and became increasingly worse as storage time extended.

Milk fortified with ascorbic acid rapidly lost its ascorbic acid when exposed to light, the rate depending upon the intensity of light and the time exposed. When exposed to direct sunlight for 40 minutes, all measurable ascorbic acid was destroyed. Stored samples in which ascorbic acid was destroyed partially developed an oxidized flavor.

Rapid destruction of all measurable ascorbic acid in milk either by exposure to direct sunlight or through the addition of 30 per cent hydrogen peroxide resulted in no oxidized flavors over a 5-day storage period, indicating that ascorbic acid is a contributing factor in the development of this off flavor. Milk in which the ascorbic acid was destroyed by the addition of hydrogen peroxide did not develop oxidized flavor when exposed to sunlight.

A study on the destruction of ascorbic acid and the development of oxidized flavor in milk under commercial conditions showed that about one-sixth of the ascorbic acid was lost from a fortified sample (30 mg. per l.) after 24

hours of storage. The same milk lost only a slight amount of ascorbic acid when carried in a case in a covered milk truck from 5 to 8 a.m. When this milk then was exposed to daylight in the shade for 30 minutes it lost about one-third of the remaining ascorbic acid. Another trial with non-fortified milk showed no loss of ascorbic acid while carried in a case on a covered milk truck for 2.5 hours. About one-half of the ascorbic acid was lost, however, when this milk was exposed to daylight in the shade for 1 hour at noontime. Exposure of the milk to direct sunlight for 15 minutes at noon destroyed all measurable quantities of the ascorbic acid. These results indicate that most of the destruction of ascorbic acid occurs after the milk leaves the truck.

ACKNOWLEDGMENTS

Roy Coleman assisted with some of the technical work in chemistry. The ascorbic acid used in these experiments was furnished by Hoffman-La Roche, Inc., Nutley, New Jersey.

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THE INFLUENCE OF TEMPERATURE OF RIPENING ON THE TYRAMINE CONTENT AND FLAVOR OF AMERICAN CHEDDAR CHEESE[1]

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It has been shown recently that a special strain of *Streptococcus faecalis,* which produced acid rapidly in milk and which grew in cheese, increased the rate of ripening and the quality of American Cheddar cheese (2, 5). This culture produced tyramine in cheese, and the amount of tyramine in both commercial and experimental cheese was directly related to the intensity of Cheddar flavor (6, 3). Within the range of usual temperatures for ripening cheese, it is known that ripening is accelerated as the temperature increases. It was the purpose of the present study to determine the influence of ripening temperatures upon the rate of production of tyramine in American Cheddar cheese made with and without *S. faecalis* starter and the usual commercial lactic starter.

EXPERIMENTAL METHODS

The Cheddar cheese was made from pasteurized milk. One batch was made on March 20, 1947, from milk produced by the herds of Cornell University, and another on April 3, 1947, from milk produced for sale as fluid milk for New York City. After mixing in the vat pasteurizer, each batch was divided into 3 parts. To one portion 2 per cent of Hansen's lactic starter was added, to another portion 2 per cent of *S. faecalis* starter was added, and to the third portion 1 per cent of lactic starter and 1 per cent of *S. faecalis* starter were added. The milk was made into cheese without giving time for the starter to develop before adding rennet, but otherwise following the time schedule of Wilson (7). All lots made up quite uniformly so the manufacturing data are not presented. The cheese was packed in tin cans containing 1 lb. of cheese sealed under 25 inches of vacuum, and the cans showed 20 to 22 inches of vacuum after sealing.

Each batch of cheese was divided into 3 lots for ripening at 40, 50 and 60° F. The cheese was placed in the curing rooms the day it was taken from the press. Samples of the cheese were analyzed for tyramine at intervals up to 1 year by the method of Kosikowsky and Dahlberg (6), for volatile acids by the method of Kosikowsky and Dahlberg (4), and for water soluble protein by the method of Sharp as reported by Dahlberg and Kosikowsky (1). The scoring was done by the authors and was discontinued after 6 months as the cheese held at 60° F. was definitely overcured in this period of time.

RESULTS

The composition of the three lots of fresh cheese was reasonably uniform and well within the range of acceptable Cheddar cheese (table 1). The fresh

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TABLE 1

Composition of the fresh cheese

	Starter used in making the cheese		
	Lactic	*faecalis*	Lactic-*faecalis*
Moisture (%)	36.07	37.74	36.16
Fat, (%)	35.0	33.5	34.5
Protein, (%)	23.61	23.65	23.40
Salt, (%)	1.62	1.55	1.67
pH	5.08	5.29	5.23

cheese made with *S. faecalis* starter only was slightly less acid and slightly higher in moisture, as *S. faecalis* starter produces acid about half as rapidly as commercial lactic starter and no correction was made for this difference by variations in the amount of starter added to the milk.

The production of tyramine in the three lots of cheese ripened at three different temperatures is given in table 2 and the flavor development in table 3. The tyramine content of cheese made from pasteurized milk with commercial lactic starter increased only slightly with ripening up to one year (table 2). The cheese milk and starter were not contaminated with bacteria that produced tyramine as indicated by the low amount of this compound in the cured cheese. Consequently, at 50° F. the ripened cheese contained only a little more tyramine than cheese ripened at 40° F., and only slightly less tyramine than cheese ripened at 60° F. All of the cheese made with commercial starter only was mild in flavor after 4 months at 40, 50 and 60° F., but after 6 months' curing at 50 and 60° F. the cheese possessed a flavor of medium intensity, even though the tyramine content was low (table 3). The flavor was not typically good Cheddar, but was flat and the cheese at 60° F. was definitely overcured

TABLE 2

Influence of temperature of ripening upon the production of tyramine in American Cheddar cheese made from pasteurized milk

Starter used in cheese	Days ripened										
	0	3	15	30	60	90	120	150	180	240	365
	Tyramine (γ/*g.*)										
	Ripened at 40° F.										
Lactic	5	.....	.....	.....	7	8	7	6	3	6	3
S. faecalis	6	.....	.....	.....	17	12	12	22	18	24	47
Lactic-*faecalis*	14	.....	.....	.....	85	57	45	77	85	120	312
	Ripened at 50° F.										
Lactic	5	.....	.....	.....	14	12	12	12	12	10	17
S. faecalis	6	.....	.....	.....	72	46	38	64	108	113	281
Lactic-*faecalis*	14	.....	.....	.....	126	163	216	280	428	534	1,369
	Ripened at 60° F.										
Lactic	5	13	14	12	19	14	16	14	17	28	37
S. faecalis	6	27	22	45	83	90	157	220	315	379	811
Lactic-*faecalis*	14	42	70	120	356	477	762	894	1,172	1,425	2,554

with a slightly burnt or caramelized taste. This interfered with judging the intensity of Cheddar flavor.

The cheese made with *S. faecalis* starter alone produced very little tyramine at 40° F. and the flavor remained mild for 6 months. When ripened at 50° F., the tyramine content of the cheese was 108 γ per g. and the flavor was medium +. The cheese ripened at 60° F. contained 315 γ per g. and the flavor was considered to be medium + in intensity.

The cheese made with both lactic and *faecalis* starters contained 85 γ per g. after 6 months at 40° F. and the flavor was mild +. Ripened at 50° F. for 6 months, the cheese contained 428 γ per g. of tyramine and the flavor was medium +. The cheese ripened at 60° F. contained 1,172 γ per g. and was sharp – in flavor intensity. The flavor was typical Cheddar of excellent quality.

Too few temperatures of ripening were employed to permit any consideration

TABLE 3

Influence of temperature of ripening upon the development of flavor in American Cheddar cheese made from pasteurized milk

Starter used in cheese	Flavor score and intensity in cheese					
	Ripened 60 d.		Ripened 120 d.		Ripened 180 d.	
	Score	Intensity	Score	Intensity	Score	Intensity
			Ripened at 40° F.			
Lactic	38.5	mild –	38.5	mild –	39.0	mild
S. faecalis	38.2	mild –	39.2	mild	39.0	mild
Lactic-*faecalis*	39.7	mild	39.5	mild	40.0	mild +
			Ripened at 50° F.			
Lactic	39.0	mild –	39.0	mild +	39.0	medium –
S. faecalis	40.0	mild	39.0	medium	39.7	medium +
Lactic-*faecalis*	40.5	mild +	40.0	medium +	40.5	medium +
			Ripened at 60° F.			
Lactic	39.5	mild +	38.5	mild +	38.0[a]	medium[a]
S. faecalis	40.0	medium	39.2	medium +	38.7	medium +
Lactic-*faecalis*	40.5	medium	40.5	sharp –	39.5	sharp –

[a] Overcured with a slight burnt or caramelized flavor, so the intensity of Cheddar flavor was difficult to judge.

of trends in the increase of tyramine production with increased ripening temperatures. However, the tyramine content of the cheese showed an approximate direct logarithmic relation to the time of ripening in days (fig. 1). The average data (fig. 1) omit all data for cheese with lactic starter only, together with all cheese stored at 40° F., as the rate of increase of tyramine was too slow to show any trend.

The data on the volatile acidity, water-soluble protein, and pH are not presented as they duplicate some previously published data (2). There was very little increase in volatile acidity in 6 months at 40° F., and this slight increase was least for the *faecalis* cheese. After 6 months the volatile acidity of the cheese was 19 ml. 0.1 *N* acid per 100 g. at 40° F. and 42 ml. at 60° F. for lactic starter cheese, which compared with 18 and 41 ml. 0.1 *N* acid per 100 g. for

lactic-*faecalis* cheese. The water-soluble protein increased about three-fold in 6 months at 40° F. The effect of increased temperature of ripening on water-soluble nitrogen after 6 months showed the lactic starter cheese contained 4.4 per cent at 40° F. and 8.1 per cent at 60° F., and the lactic-*faecalis* cheese contained 4.3 and 8.4 per cent, respectively, of water-soluble protein.

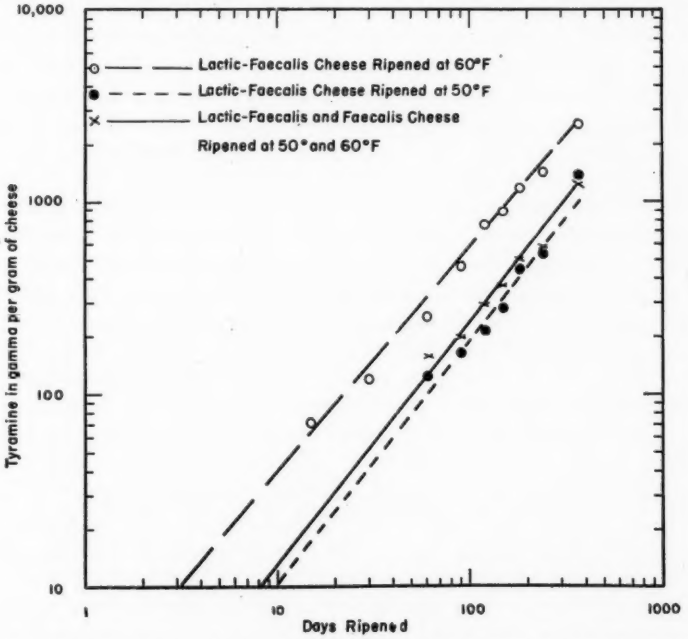

FIG. 1. The rate of tyramine production in cheese made with *S. faecalis* starter alone, and with commercial lactic and *faecalis* starters.

DISCUSSION

The amount of tyramine in the cured cheese made from pasteurized milk with commercial lactic starter alone was especially low as compared with the tyramine previously found in commercial Cheddar cheese (6). Undoubtedly, this pasteurized milk of high sanitary quality did not contain tyramine-producing bacteria or they may have been present in very small numbers. The cheese developed normal acidity, volatile fatty acids, and water-soluble protein during ripening, but the intensity of flavor remained mild and was not increased greatly by warmer ripening temperatures. This illustrates indirectly previous results (3) that increased tyramine and Cheddar flavor intensity are related. Limited flavor developed with ripening and with very little tyramine production, which shows that all flavor is not associated with tyramine, but most of the typical Cheddar flavor is related to the presence of tyramine.

S. faecalis alone in cheese increased tyramine production, but the effect was

very limited at 40° F. It should be observed that the temperatures of this refrigerator varied from 35 to 42° F. and was generally slightly below 40° F. *S. faecalis* grows poorly at this temperature. Ripening at 50 and 60° F. gave marked increases in tyramine and obvious increases in the intensity and quality of flavor. *S. faecalis* grows well at these warmer temperatures.

The use of regular lactic and *faecalis* starters in pasteurized milk markedly increased tyramine production in cheese at all temperatures of curing, including 40° F. The intensity of Cheddar flavor and the quality of the Cheddar flavor were best in the cheese made with both starters.

It should be pointed out that the percentage of starter added to the milk in these experiments was rather large, but similar results have been observed with variations from 0.1 to 2.0 per cent of the *faecalis* starter.

On several occasions it has been observed that Cheddar cheese made with *S. faecalis* starter not only developed flavor more rapidly but this flavor was maintained for longer periods of time than in cheese without this culture. This was observed in cheese cured at 60° F. The cheese made with lactic starter alone decreased progressively in score with increased age and the flavor intensity increased slightly. At 180 days this cheese was overcured and had an off-flavor. The cheese made with both lactic and *faecalis* starters was sharp – in intensity at 120 days, yet it was held for 180 days without developing an off-flavor. The best keeping quality was shown by the cheese made with both starters.

It is believed that 40° F. or below is too cold a temperature for ripening Cheddar cheese made from pasteurized milk of good quality with both starters to obtain maximum development of good Cheddar flavor in a reasonable period of time. An initial curing for 2 months at 60° F. or 4 months at 50° F. will develop good pronounced Cheddar flavor and then the cheese properly made may be stored a long time at 40° F. or below with assurance of excellent flavor.

In considering the data on flavor, it should be borne in mind that flavor intensity was rated over a 6-month period. Consequently, there is more chance for variation in judgement than if all samples were rated at one time.

This research emphasizes that the value of *S. faecalis* starter in ripening cheese will be greatest in milk of highest sanitary quality. Its value will be least in milk of low bacterial count but relatively high in normal infection with *S. faecalis* or other tyramine-producing bacteria, and in milk of lower sanitary quality which normally contains these bacteria in substantial numbers. When these bacteria were absent, force curing at a high temperature, such as 60° F., accelerate flavor development to some extent but not enough to obtain a full Cheddar flavor.

There is an indication that the rate of development of tyramine in the early days of ripening might be used as an index to predict whether cheese will cure slowly or rapidly. More data are necessary to establish this point.

CONCLUSIONS

The pasteurized milk used for making the experimental American Cheddar cheese was practically free from tyramine-producing bacteria, as indicated by

the tyramine content. After 6 months ripening at 40, 50 and 60° F., the tyramine content of cheese made with commercial lactic starter was only 3, 12 and 17 γ per g. The flavor was mild, medium – and medium in intensity and lacked good Cheddar characteristics.

When *S. faecalis* starter (special rapid acid-producing strain) was used alone in the pasteurized milk, the tyramine content of the cheese after 6 months ripening at 40, 50 and 60° F. was 18, 108 and 315 γ per g. The flavor intensities were mild, medium – and medium +, respectively.

The use of both commercial lactic and *S. faecalis* starters in the pasteurized milk produced the greatest development of tyramine and good, typical Cheddar flavor. After 6 months' ripening at 40, 50 and 60° F. the tyramine content was 85, 428 and 1172 γ per g. and the flavor intensity was mild +, medium + and sharp –. The sharp – intensity of flavor was obtained in 4 months.

Cheddar cheese containing *S. faecalis* developed more tyramine and Cheddar flavor as the ripening temperatures were increased. It maintained its good flavor for a longer period of time without becoming overcured at warm temperatures than did cheese made only with ordinary lactic starter. The increase in the tyramine content of cheese showed an approximately direct logarithmic relation to the days ripened.

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EFFECT OF THYROXINE ON FERTILITY OF BOVINE SEMEN[1]

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The addition of proper amounts of D,L-thyroxine to bovine semen appears to exert an influence on sperm metabolism (1, 2). It is logical to suppose that a change in metabolism influences semen fertility. Therefore this study was undertaken to determine whether bovine semen fertility is influenced by the addition of thyroxine. Since the over-all fertility of bovine semen obtained under artificial breeding conditions depends both on the initial fertility and the maintenance of a high level of fertility during storage, the data were studied to determine whether thyroxine influences the initial fertility, the maintenance of fertility or both of these characteristics.

METHODS

D,L-thyroxine was added to egg yolk-phosphate-buffered bovine semen in a concentration of 10 γ per 100 ml. diluted semen. A stock solution of the thyroxine was prepared by weighing 10 mg. of the material and dissolving it slowly and completely in distilled water by the addition of 0.1 *N* NaOH drop by drop. When dissolved the solution was made up to 100 ml. volume. The thyroxine solution added to the diluted semen was prepared from this stock solution by adding phosphate buffer in measured volumes so that the buffered thyroxine solution contained 5 γ thyroxine per ml. All thyroxine solutions were stored at 40° F. Portions of this latter buffered solution were added to the egg yolk-phosphate-buffered semen so that the final concentration of thyroxine was 10 γ in 100 ml. diluted semen.

Semen samples were selected at random from 18 bulls in the University stud and treated with thyroxine. Other semen samples from the same bulls during the same periods of time served as controls. Non-return rates obtained from the use of such a series of semen samples vary from that obtained with another series even where semen are from the same bulls during the same season. The variation among several such series involving semen sample numbers and service numbers approximating those of the thyroxine-treated semen in our experiments appeared not to exceed 2 percentage units in non-return rate. To compare the results of treatment on the same semen sample, an experiment was carried out in which one portion of a semen sample was treated with thyroxine and sent to one group of associations for use; the other portion, untreated, was sent to a second group of associations. The two association groups had similar non-return rates when both used untreated semen from the same bulls.

Breeding results are based on 5 months' non-return rates of first and second services in routine artificial breeding.

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RESULTS

The experiment to determine the effect of thyroxine on fertility of bovine semen was carried out over a 2-year period. In the first part of the experiment a portion of randomly selected semen samples was treated with thyroxine and sent to group 1 associations for use. The other portion of these semen samples was left untreated and sent to group 2 associations. More than 50 individual semen samples were used in this part of the experiment. The results showed that the non-return rate for the treated semen in group 1 (422 services) was 59.1 per cent and that for the untreated portion of the samples in group 2 associations (456 services) was 54.4 per cent, a difference of 4.7 percentage units. However, when the semen sent to both groups 1 and 2 was all untreated and from the same bulls, the non-return rate for the group 1 associations was 1.2

TABLE 1

Non-return rate with treated and control semen with age

	Days storage								All days combined	
	1		2		3		4			
	No. services	5-mo. non-returns	No. services	5-mo. non-returns	No. services	5-mo. non-returns	No. services	5-mo. non-returns	No. services	5-mo. non-returns
		(%)		(%)		(%)		(%)		(%)
Semen treated with thyroxine	384	65.8	821	66.1	496	62.5	166	60.2	1887	64.6
Control semen	1303	63.1	2470	59.5	1489	56.0	667	52.1	5929	58.5
Increase over control		2.7		6.6		6.5		8.1		6.1

percentage units lower than for the group 2 associations. Therefore, there appeared to be a net increase of 5.9 percentage units in non-return rate resulting from the presence of thyroxine in the semen.

The experiment was extended by sending semen samples treated with thyroxine to all associations. The non-return rate obtained with treated semen was compared with that obtained with control semen from the same bulls during the months in which semen was treated. The samples used as controls were from those used before or after the treated samples. The non-return rate from 92 treated semen samples with 1,867 services involved was 64.6 per cent. The non-return rate from untreated control semen samples was 58.5 per cent. Thus the addition of thyroxine appeared to increase the non-return rate by 6.1 percentage units.

A comparison of the non-return rates from treated and control semen with days of storage is shown in table 1. It is apparent that fertility was maintained

TABLE 2

Comparison of non-return rate of thyroxine-treated and control semen by individual bulls[a]

Semen samples	Bull	Treated		Control		Difference
		Services	5-mo. non-returns	Services	5-mo. non-returns	
	(*No.*)	(*No.*)	(%)	(*No.*)	(%)	(*Percentage Units*)
1	5	66	56.1	136	52.2	+ 3.9
2	2	36	75.0	112	52.7	+22.3
3	6	95	47.3	307	50.2	− 2.9
4	6	91	62.6	228	57.5	+ 5.1
5	1	20	85.0	198	60.1	+24.9
6	9	201	62.7	347	60.8	+ 1.9
7	7	235	65.1	463	59.8	+ 5.3
8	4	128	66.4	179	58.1	+ 8.3
9	5	97	63.9	231	54.1	+ 9.8
10	6	180	65.6	254	60.4	+ 5.2
11	1	22	63.6	193	59.5	+ 4.1
12	7	80	68.7	200	56.5	+12.2
13	13	185	66.5	391	60.1	+ 6.4
14	6	76	68.4	268	64.9	+ 3.5
15	1	35	62.8	193	67.4	− 4.6
16	2	40	75.0	89	68.5	+ 6.5
All	81	1587	64.5	3789	58.8	+ 5.7

[a] Bulls having less than 20 treated services omitted from table.

at a higher level throughout the 4 days of use. The difference during the first day of use is less than with increased storage, indicating a delayed thyroxine effect.

The data were studied by grouping semen samples by individual bulls. The comparison is shown in table 2. Semen samples from bulls used in less than 20 services have been omitted from the tabulation. In evaluating the average

TABLE 3

Comparison of non-return rate with thyroxine-treated and control semen by individual breeding associations. (Only services with same storage time for control and treated semen considered.)

Association	Treated		Untreated		Difference
	No. services	5-mo. non-returns	No. services	5-mo. non-returns	
		(%)		(%)	(*Percentage Units*)
A	320	60.3	308	55.8	+ 4.5
B	256	65.6	264	61.7	+ 3.9
C	86	69.8	114	56.9	+12.9
D	136	39.0	132	35.6	+ 3.4
E	98	73.5	99	61.5	+12.0
F	122	62.3	146	55.8	+ 6.5
G	107	60.7	95	53.6	+ 7.1
H	74	82.4	79	69.6	+12.8
I	115	73.0	121	63.6	+ 9.4
J	249	63.9	250	55.8	+ 8.1
All associations	1563	63.4	1608	56.7	+ 6.7

non-return rate for all bulls, the non-return rate percentage of each bull was weighted in proportion to the number of treated services so that the percentage influence of each bull's services on the total results would be the same for both treated and untreated semen. On this basis of comparison, thyroxine increased the conception rate 5.6 percentage units. The semen from two bulls showed a decrease in fertility due to treatment; however, in one of these bulls only one semen sample represented the treated services.

In a comparison of treated and control semen by individual associations, the controls were recorded only for those days on which the treated semen was used, to eliminate insofar as possible the effect of semen storage on the comparison. Results are shown in table 3. The non-return rate in all associations was increased by treatment and the difference for all associations was found to be 6.7 percentage units. This percentage unit increase is somewhat higher than that found with other bases of comparison; however, it is not significantly different.

To test whether the difference in non-return rate due to treatment was statistically significant, the results were analyzed on an individual semen sample basis for both treated and control samples. Semen samples with less than ten services were eliminated. The non-return rate percentages were converted to angles. The difference between treated and control samples was found to be significant at less than the 5 per cent level. When the variance between individual semen samples was eliminated, significance was at less than the 1 per cent level.

DISCUSSION

It appears from the data at hand that 10 γ per cent D,L-thyroxine when added to diluted bovine semen increases the fertility. This is apparent with semen from bulls of average or above average fertility. The difference in fertility is small for the first day of storage but increases to a maximum the second day of storage. This increase in fertility apparently is maintained for a 4-day storage period. The non-return rate for treated semen was lower than that for control semen with the semen of one bull. This bull's average fertility was relatively low and it is possible that thyroxine in the amount used had a detrimental effect on this poor semen. A limited number of thyroxine-treated semen samples from bulls with less than 50 per cent non-return rate was available for study. It seems from these limited data that semen low in fertility is increased in non-return rate above that of control semen of low fertility during at least 2 days of storage, but declines below that of the control level by the 3rd or 4th day of storage. Semen lower in fertility than that represented by the data at hand may be more unfavorably affected by storage.

The selection of semen samples used for controls during the period of the experiment was varied so that accidental selection of better than average or poorer than average semen from the bulls on experiment would be evident if this were affecting the results. The selection of different control semen samples resulted in a difference of 2 percentage units in the control values. This is a non-significant difference and can probably be expected by random selection of

semen from the same bulls during the same periods of the year with the number of services and semen samples involved.

SUMMARY

Ten micrograms per cent of D,L-thyroxine was added to diluted bovine semen. This semen was used in artificial breeding.

All treated services (2,289) averaged 5.7 to 6.7 percentage units higher in percentage of non-returns to service after 5 months than untreated semen from the same bulls collected during the same months that the treated semen was used. In a comparison of either individual bulls or individual breeding associations, an increase in non-return rate is also apparent with thyroxine-treated semen.

The increase in fertility with the addition of thyroxine appears to be maintained for a 4-day storage period with semen of average or above average fertility.

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THE EFFECT OF TESTIS BIOPSY ON SEMEN CHARACTERISTICS OF BULLS[1]

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Biopsy technics have been used by many investigators to study histological changes in various living organs. Erb *et al.* (2) in a preliminary report have described a biopsy technic by means of which they correlated semen quality with testis morphology in dairy bulls during vitamin A studies. We have used this same technic in experiments on the effects of energy restriction on semen characteristics of bulls, and during the course of these experiments evidence was obtained that this biopsy technic produced changes in semen characteristics and in testis morphology which were unrelated to dietary conditions.

MATERIALS AND METHODS

Studies were made on three mature bulls. When the first biopsy specimen was taken, all were just past 6 years of age. Two of the bulls (no. 1 and 2) had been on a ration which restricted energy intake to 65 per cent of requirement, up to the time the first specimen (from the left testis only) was taken. Feed intake then was raised to 100 per cent of requirement and maintained at that level until the bulls were disposed of. A specimen was obtained (from the right testis) on one of these bulls (no. 2) 6.5 months later and on the other (no. 1) 10.5 months later. Both bulls were castrated 3 months after the second biopsy and representative sections of testis tissue were obtained.

The first specimen (from the left testis) was obtained from the third bull (no. 3) immediately before the ration was reduced to the 65 per cent energy-intake level. He remained on this level of energy-intake for 15 months, and a second specimen (from the right testis) then was obtained. Feed intake then was increased to 100 per cent of requirements, and the bull was castrated 3 months later.

The biopsy technic performed under local anesthesia was essentially that described by Erb *et al.* (2), with the exception that hemorrhage always was controlled by applying manual pressure to the spermatic artery until a solid clot had formed. The tunica albuginea and skin incision always were sutured. Recovery was rapid and uneventful in every instance.

Semen was collected from the bulls by means of an artificial vagina. With only occasional omissions, four ejaculates were collected weekly from each bull during the period of observation, two ejaculates being obtained in succession at each of two services. Motility determinations were made on undiluted semen

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in a stage warming apparatus. Sperm counts and the determination of abnormal spermatozoa were made with a hemocytometer on fresh semen diluted with a 0.9 per cent saline solution. The data presented here all are based on the second ejaculate.

Tissue sections were stained with hematoxylin and eosin for general morphological examination and with Mallory's aniline blue connective tissue stain to detect the relative amounts of connective tissue in the various specimens.

RESULTS

The data obtained on semen characteristics are shown for each of the three bulls in table 1. It will be noted that each biopsy produced a marked decrease

TABLE 1

Changes in sperm characteristics as the result of biopsy

Period of observation	Sperm count	Initial motility	Storage motility[1]	Percentage of abnormal sperm cells	Volume	Number of observations
	(millions/ml.)	*(%)*	*(%)*		*(ml.)*	
Bull no. 1						
Control (60 days)	1360	54.3[2]	9.9[2]	63.9[2]	3.4	7
Biopsy specimen obtained—returned to adequate feed intake						
(60 days)	700	85.0	17.5	33.5	3.8	4
(274 days)	639	67.9	27.6	34.0	5.0	27
Second biopsy (no change in feed)						
(30 days)	653	30.3	7.0	37.0	4.4	3
(60 days)	388	44.8	8.8	28.2	5.8	8
Bull no. 2						
Control (60 days)	1012	70.0	40.0	27.7	2.8	9
Biopsy specimen obtained—returned to adequate feed intake						
(60 days)	862	72.5	25.5	24.6	4.2	5
(142 days)	556	73.0	30.8	20.7	4.4	13
Second biopsy (no change in feed)						
(30 days)	723	38.3	20.0	45.0	4.6	3
(60 days)	264	46.3	11.9	32.6	4.2	9
Bull no. 3						
Control (60 days)	841	86.2	48.1	18.9	4.7	8
Biopsy specimen obtained—feed intake reduced						
(50 days)	458	74.5	26.5	41.0	5.5	5
(376 days)	465	76.0	31.7	38.8	4.3	41
Second Biopsy—returned to adequate feed intake						
(30 days)	125	5.0	2.0	75.2	2.7	4
(60 days)	0.13	0	0	82.0	3.7	7

[1] Storage motility = motility after 48 hrs. storage at 4–6° C.

[2] These low motility values and the high percentage of abnormal sperm cells resulted from restriction of feed intake.

in sperm concentration in all three bulls, which roughly amounted to 50 per cent except in the case of the second biopsy on bull no. 3. Sperm production practically ceased following the second biopsy in the case of bull no. 3. The

full effect of a biopsy was not always immediately evident, and for this reason the period of observation following each biopsy has been divided into a short and a longer interval. Usually the first two or three ejaculates obtained contained numbers of sperm characteristic of the pre-biopsy period. This was at least partly due to the fact that a 2-week period of sexual rest was usually allowed after each biopsy. The decrease in sperm concentration which followed this short interval persisted throughout the remainder of the observation period in all cases, and there has been no indication that improvement in spermatogenesis occurred even though the periods of observation after the first biopsy was performed ranged from 202 to 426 days.

It also will be noted that changes were made in the ration of these animals in every instance at the time the first biopsy specimen was obtained. In the case of bull no. 3, feed intake was reduced at this time. During the following 14-month period, his weight loss amounted to 293 lb. The change in sperm concentration possibly could be accounted for by the poor nutritional condition of this bull. However, a much greater decrease in sperm concentration occurred in the semen of this bull when the second biopsy was performed, even though feed intake was increased to normal at this time. Moreover, this change was noted in the semen of bulls no. 1 and no. 2 after both biopsies. The first biopsy was performed on these bulls at a time when feed intake was increased and the bulls thereafter were improving in condition. The second biopsy was performed on these bulls while they were on normal and constant feed intakes. The change in sperm count was just as great as under the previous regime. It seems evident, therefore, that the change in sperm concentration was related to the biopsies rather than to changes in the ration which coincided with the biopsies in most instances.

It also is evident that a serious misinterpretation of experimental results might have occurred if all three of these bulls had been biopsied just previous to the time that the restriction in energy intake was imposed. It would not have been illogical to ascribe the decrease in sperm production to the reduced energy intake rather than to the effects of biopsy. The data presented by Erb *et al.* (2), in the paper in which they described the biopsy technic, show that a marked drop in concentration of spermatozoa occurred after each of the biopsies that were taken from the bull used in their work. The decrease in concentration of spermatozoa after the first biopsy was ascribed to a gradual depletion of stored sperm. No comment was made on the decrease after the second biopsy.

In view of our results, it would appear that the semen change reported by Erb *et al.* (2) was related more to the biopsies than to the vitamin A status of the animal. It also will be noted that improvement in semen occurred following the initial drop in sperm concentration after each biopsy on the bull described by Erb *et al.* (2). This effect has not been seen in the present work and this difference in results between the two studies may be due to the differences in age of the bulls used.

The effect of the biopsies on motility and on the percentage of abnormal cells was somewhat variable. The first biopsy on bulls no. 1 and 2 did not decrease motility or increase the number of abnormal spermatozoa. The increase in motility and the decrease in abnormal cells which occurred after the first biopsy, in the case of bull no. 1, is believed to be due to the improved nutritional status of the bull during this interval. Attention is drawn to the fact that the control values for this bull (see table 1) were abnormal and a result of the previous feed restriction. In the case of bull no. 3, however, the first biopsy did decrease motility and increase the percentage of abnormal cells. The fact that no pro-

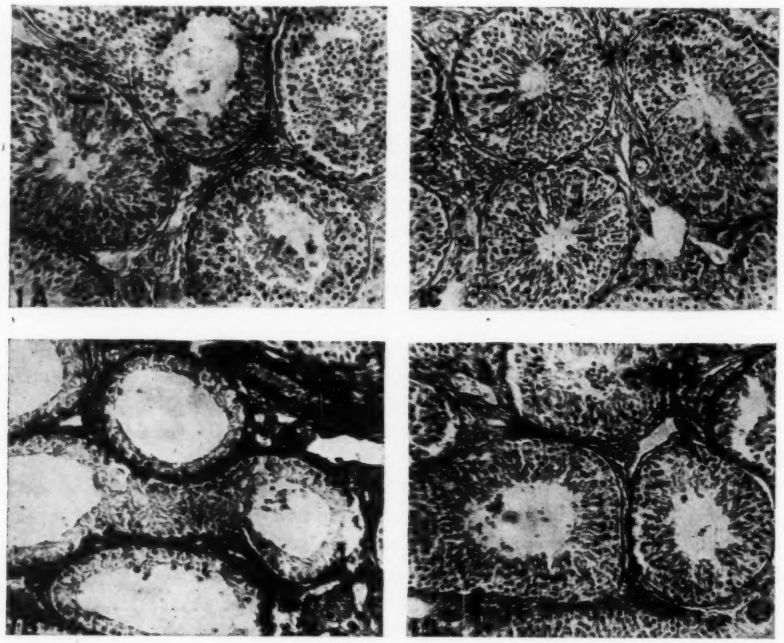

FIG. 1. Comparison of biopsy specimens of testis and specimens obtained on the same testis after castration. X200.

1A—Right testis biopsy specimen.

1B—Right testis at time of castration.

1C—Left testis biopsy specimen.

1D—Left testis at time of castration.

gressive change in these characteristics occurred in the succeeding period of feed restriction would indicate this was an effect of the biopsy and not a result of feed restriction. In all cases, however, the succeeding biopsy on the opposite testis markedly reduced motility and increased the percentage of abnormal cells. The data therefore would indicate that a single biopsy on one testis may or may not affect the motility and normality of the sperm cells but that a second biopsy, even when obtained from the opposite testis, has a definite and adverse effect on the motility and normality of sperm cells. Bogart and Mayer (1) noted that

similar changes in semen characteristics occurred in sheep after removal of one testis. Moore (3) also has noted that adhesions occur around the testis of bulls following inflammation due to infection or trauma of the testis and are accompanied by a progressive deterioration of semen quality. In the three bulls in the present study, marked adhesions between the tunica vaginalis and tunica albuginea were noted when the bulls were castrated.

The photomicrographs shown in figure 1 are representative of the morphological changes noted in the testis tissue as determined at castration. A comparison of figure 1A and 1B illustrates the marked change which occurred in the right testis following biopsy. Figure 1A, from tissue taken at biopsy, shows there was some disorganization and desquamation of tissue and that sperm cells were not numerous in the tubules. Figure 1B shows the condition of the same testis 3 months later. Sperm cells were practically nonexistent and spermatogenic tissue was reduced markedly. This tissue, which was obtained at castration, was representative of the general condition throughout the testis except in the case of bull no. 3. In this bull, while spermatogenesis was reduced, less than 25 per cent of the tubules showed this marked degeneration, although most tubules showed degenerative changes when compared to the biopsy specimens obtained previously.

Figure 1C represents the biopsy specimen and figure 1D the tissue obtained at castration from the left testis. It will be noted that there is little or no difference in the appearance of the tissues. Two of the bulls (no. 1 and 2) reacted in this manner. The left testis of the third bull, however, showed very marked degeneration at the time of castration. The testis was about $\frac{1}{3}$ normal size and the upper third was distinctly calcified. Histologically, degeneration as marked as that shown in figure 1B was present throughout the testis and the tubules in the calcified area were filled with mineral deposit.

It is difficult to account for the variability in reaction to biopsy between the right and left testis of these animals. In all cases the interval of time between biopsy of the right testis and castration was 3 months. The intervals between biopsy of the left testis and castration were 14, 10 and 17 months for bulls no. 1, 2 and 3, respectively. In the case of bulls no. 1 and 2, the presence of marked degeneration in the right testis 3 months after biopsy and little or no change in the left testis 10 or 14 months after biopsy might indicate that repair had occurred in the left testis during the longer time interval. If repair did occur in the testis of these two bulls, it was unaccompanied by any improvement in spermatogenesis as previously noted. The marked degeneration noted in the left testis of bull no. 3 at castration, even though an interval of 17 months elapsed between biopsy and castration, is in direct contrast to the other two bulls. Bull no. 3 lost considerable weight (293 lb.) during 14 of the 17 months of this interval of time due to restriction of feed intake, whereas bulls no. 1 and 2 had adequate feed and were gaining in weight throughout most of this period. It therefore is possible that the reduced feed intake of bull no. 3 intensified the biopsy effect and was responsible for the difference in effects on the left testis observed between this bull and bulls no. 1 and 2.

As mentioned previously, rather extensive adhesions between the tunica vaginalis and tunica albuginea were found at castration. Marked connective tissue proliferation, of course, was present at the site of biopsy in every instance. In addition to these changes, an increase in the amount of connective tissue throughout the intertubular spaces of both testes was noted in the tissues obtained when the bulls were castrated. This reaction was not as intense as that observed at the biopsy site, but in every instance a distinct increase was noted in the amount of intertubular connective tissue as compared with the amount in the original biopsy specimens. This increase in connective tissue was roughly

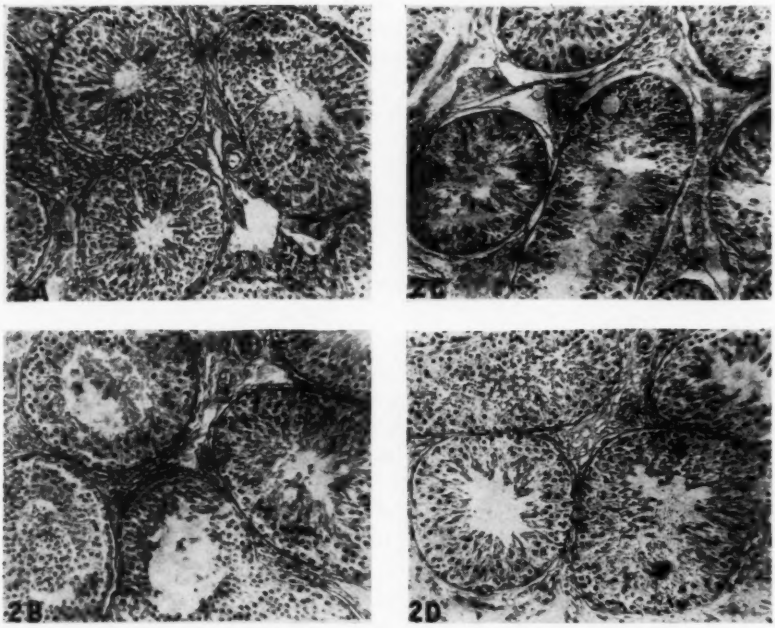

FIG. 2. Comparison of right and left testis biopsy specimens from bulls no. 2 and no. 3. X200.

2A—Left testis specimen (bull no. 2).
2B—Right testis specimen (bull no. 2).
2C—Left testis specimen (bull no. 3).
2D—Right testis specimen (bull no. 3).

proportional to the degree of tubular degeneration observed. It was greatest in the left testis of bull no. 3 and in the right testis of bulls no. 1 and 2 in which the greatest tubular change was observed and, while definitely increased, was less evident in the right testis of bull no. 3 and the left testis of bulls no. 1 and 2. It therefore would appear that the biopsies produced a marked proliferation of connective tissue throughout the testis and that this change was related to the morphological and functional changes which were observed in the tubules.

If the increased collagen noted in these sections was a result of a fibroblastic

reaction, this reaction had subsided by the time sections were obtained. However, there was some evidence that the numbers of interstitial cells were increased in those specimens which showed increases in connective tissue.

The evidence at our disposal did not suggest that a biopsy on one testis had any marked effect on the opposite testis. A comparison of tissues obtained at successive biopsies of the left and right testis is shown in figure 2 to illustrate this point. Tissue from the left testis biopsy (2A) and tissue from the right testis (2B) obtained by biopsy 6 months later from bull no. 2 is shown. Comparison of these tissues indicates that possibly some change occurred in the right testis as a result of biopsy on the left testis. There also was some slight indication of an increase in connective tissue in the right testis. A comparison of figures 2C and 2D, which are similar tissues from bull no. 3, shows no difference. A like comparison between the biopsy specimens from the left and right testis from no. 1 indicated no effect other than some increase in connective tissue in the right testis at biopsy. The intervals between biopsies in the case of bulls no. 1 and 3 were 11 and 14 months, respectively, whereas it was only 6 months in the case of bull no. 2. If the difference noted between figures 2A and 2B really indicates that the biopsy of the left testis affected the opposite testis of bull no. 2, and that similar changes were produced in the right testis of bull no. 1 and bull no. 3, it might be assumed that the longer interval between biopsies in the case of the latter two bulls gave sufficient time for repair to take place so that no evidence of damage was present at the succeeding biopsy. In general, however, the direct evidence available would not indicate that biopsy on one testis produced morphological changes in the other testis.

SUMMARY AND CONCLUSIONS

1. Biopsy of the testis of mature bulls resulted in a marked decrease in sperm concentration in the semen.

2. Biopsy of a single testis had variable effects on motility or percentage of abnormal sperm, but a succeeding biopsy on the second testis decreased motility and increased the percentage of abnormal sperm, and in one bull resulted in almost complete absence of spermatogenesis.

3. Marked morphological changes were observed in the tubules of the testis and in the amount of intertubular connective tissue. In one instance, marked atrophy of the testis occurred as a result of biopsy.

4. These data would indicate that considerable care must be used in interpreting data in experimental studies on semen production and semen characteristics where biopsies are performed in conjunction with other nutritional or physiological technics.

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SOME FACTORS INFLUENCING THE REDUCING SYSTEMS IN DRY WHOLE MILK[1]

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The first extensive data relating the heat-labile sulfur of milk to cooked flavor and antioxygenic activity were obtained by Gould and Sommer (7) and Josephson and Doan (13). The former collected the heat-labile sulfur with lead acetate paper and found that heat treatments sufficient to cause cooked flavors and a lowering of the oxidation-reduction potential also produced volatile sulfur. Very similar results were obtained by Josephson and Doan (13) using the nitroprusside test for heat-labile sulfur.

The antioxygenic properties of dry whole milk manufactured from milk preheated to a high temperature have been ascribed to the sulfur-containing reducing groups (10, 11). Mattick *et al.* (15) were the first to show that the presence of nitroprusside-reducing substances resulting from the preheating treatment of the milk is associated with increased resistance to oxidation of the dry milk during storage.

The effect of several preheating temperatures on the keeping quality of the dry whole milk was studied by Findlay *et al.* (6). Preheating temperatures producing antioxygenic effects in the powder were those yielding positive nitroprusside and volatile sulfur tests. No data were reported concerning the effect of subsequent oxidation of the dry whole milk during storage on the sulfhydryl groups initially present.

A method for the estimation of sulfhydryl groups in blood based on reduction of thiamin disulfide has been adapted to milk by Harland and Ashworth (9). The experiments described herein were undertaken to test the thiamin disulfide reagent for the estimation of the antioxygenic qualities of dry whole milk, prepared from milk preheated to high temperatures, to determine the source of the groups that reduce this reagent and to compare the method with other means of measurement of reducing substances in milk.

METHODS

Thiamin disulfide reducing substances (TDRS) were determined essentially according to the method of Harland and Ashworth (9). Acid ferricyanide reducing substances (AFRS) were estimated by the Chapman and McFarlane procedure (2) as modified by Crowe *et al.* (4). Ascorbic acid (apparent) was estimated by the method of Doan and Josephson (5). Solubility indices of the dry whole milk were performed according to the method of the American Dry Milk Institute (1).

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Drying of the skim milk and the whole milk used in this work was accomplished in an experimental spray dryer of the University of Minnesota. Milk flow was regulated to secure an exit air temperature of 85° C. for what was considered to be a normal drying temperature and 104° C. for a high drying temperature.

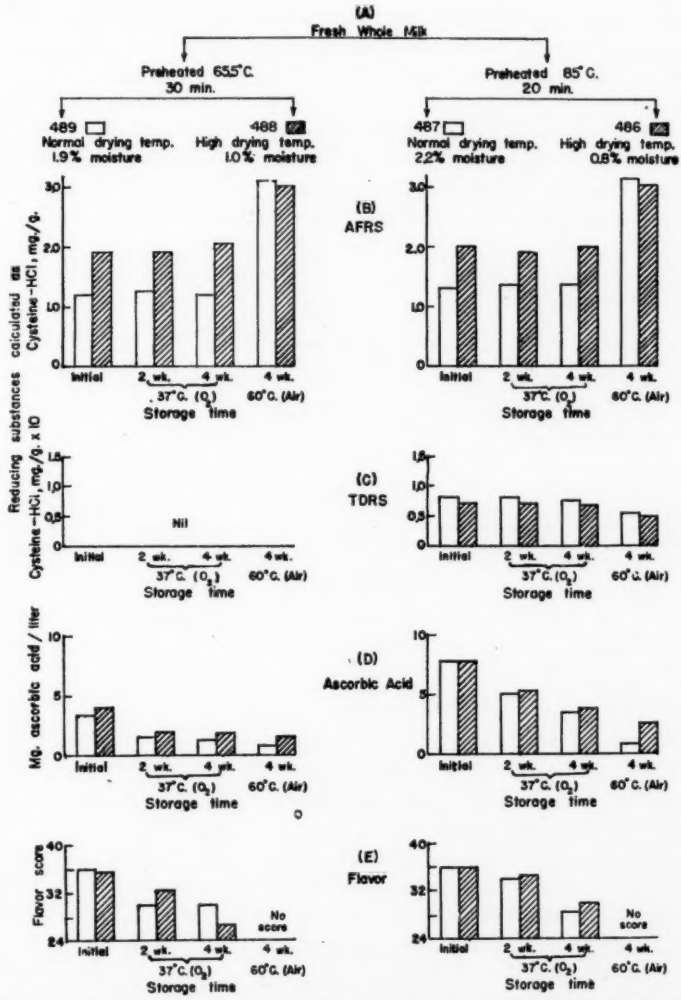

FIG. 1. The effects of preheating and drying temperatures on the acid ferricyanide (AFRS) and thiamin disulfide (TDRS) reducing substances, ascorbic acid, and keeping quality of dry whole milk. Similar results were obtained from a duplicate experiment.

Heat treatments of the liquid systems were done in thin-walled, 8-inch stainless steel test tubes of 20 ml. capacity immersed in a rapidly-circulating water

bath maintained at constant temperature. Deaeration of the liquid systems was accomplished by a brief period of boiling, under a pressure of approximately 25 mm. of mercury, and flooding the system with commercial nitrogen. This process was repeated three times before heating in an atmosphere of nitrogen.

Scoring of the dry whole milk was done according to the student score card.

Nonfat dry milk solids were prepared from freshly separated milk of good quality preheated at 63° C. for 30 minutes. Dry whole milk was prepared from good quality fresh milk and had solubility indices not exceeding 0.1. Heat treatments were as indicated in the data. Casein was prepared as previously reported (12) and dispersed in a minimum quantity of 0.1 *N* NaOH to pH 6.6 to obtain the sodium caseinate. Serum proteins were prepared by removal of the casein from reconstituted nonfat dry milk solids with acetate buffer at pH 4.5, concentration of the whey by freezing, exhaustive dialysis in Visking sausage casings and further concentration by pervaporation[2] from the casing. This preparation was stored under toluene at approximately 4° C. until used. Lactose was of U.S.P. quality.

RESULTS

Relation of reducing substances to retention of ascorbic acid and flavor during storage. A preliminary experiment was designed to indicate some of the re-

TABLE 1

Composition of the simplified systems used for heat treatment

System	Casein (as Na Caseinate)	Milk serum protein	Lactose	Phosphate buffer pH 6.6
	(*g./100 ml.*)	(*g./100 ml.*)	(*g./100 ml.*)	(μ)
1	2.50	.....	.....	0.10
2	2.50	.....	4.75	0.10
3	.....	0.70	.....	0.10
4	.....	0.70	4.75	0.10
5	Reconstituted nonfat dry milk solids 10 g./100 ml.			

lationships of reducing capacity to the conditions of manufacture and storage of dry whole milk and to determine whether retention of ascorbic acid and flavor during storage is related to reducing capacity. The experimental plan as well as the data are presented in figure 1.

The acid ferricyanide reducing substances (AFRS) (fig. 1B) were not influenced significantly by the preheating temperatures used, but were increased by the higher drying temperature. This is in agreement with results reported in a recent paper (4). Although storage of the powder at 37° C. for periods up to 4 weeks had little effect on the AFRS, storage at 60° C. caused a large increase in these substances.

The preheating temperature was the predominant factor influencing the thiamin disulfide reducing substances (TDRS) (fig. 1C). The apparent de-

[2] Evaporation of water from the sol through the membrane. (Kober, P. A., Pervaporation, Perstillation, and Pererystallation. J. Am. Chem. Soc., 39: 944–48. 1917.)

crease in these substances during storage of the dry whole milk at 60° C. for 4 weeks could not be substantiated when this experiment was repeated. High drying temperatures appear to depress slightly the TDRS produced in the milk by heat treatment.

The protective action of the higher preheating temperature on the ascorbic acid content of the dry whole milk both during drying and in subsequent storage is apparent in figure 1D. The high drying temperature also seems to afford some degree of protection for this easily oxidized substance.

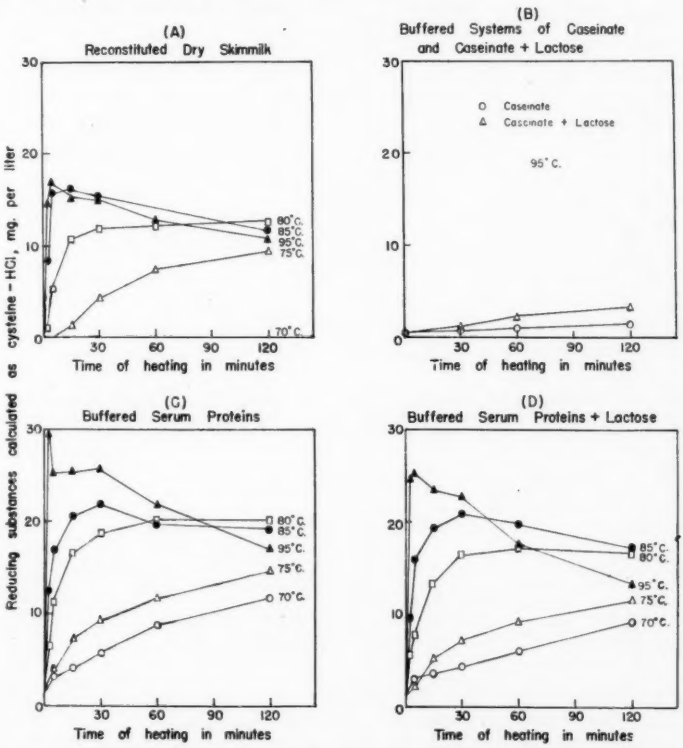

FIG. 2. The effect of heat treatment of skim milk and simplified systems on production of thiamin disulfide reducing substances. The points shown in these graphs are the average of at least two values.

The flavor scores (fig. 1E), although typically rather erratic, show some increased retention of quality during storage as the result of the higher preheating temperature.

Source of TDRS and AFRS produced during heat treatment. It is believed generally that the sulfhydryl groups liberated in milk during heat treatment originate from the proteins and, more specifically, the serum proteins. To obtain information on this point skim milk (actually reconstituted nonfat dry milk

solids) and various simplified systems, the composition of which is tabulated in table 1, were heat-treated at various temperatures for periods up to 120 minutes. Reducing power was determined in these systems by both the thiamin disulfide and acid ferricyanide methods. Since during preliminary trials the oxygen tension of milk had been found to affect the production of TDRS, the systems studied in this experiment were deaerated before beginning the heat treatments. The data for TDRS and AFRS are shown in figures 2 and 3, respectively.

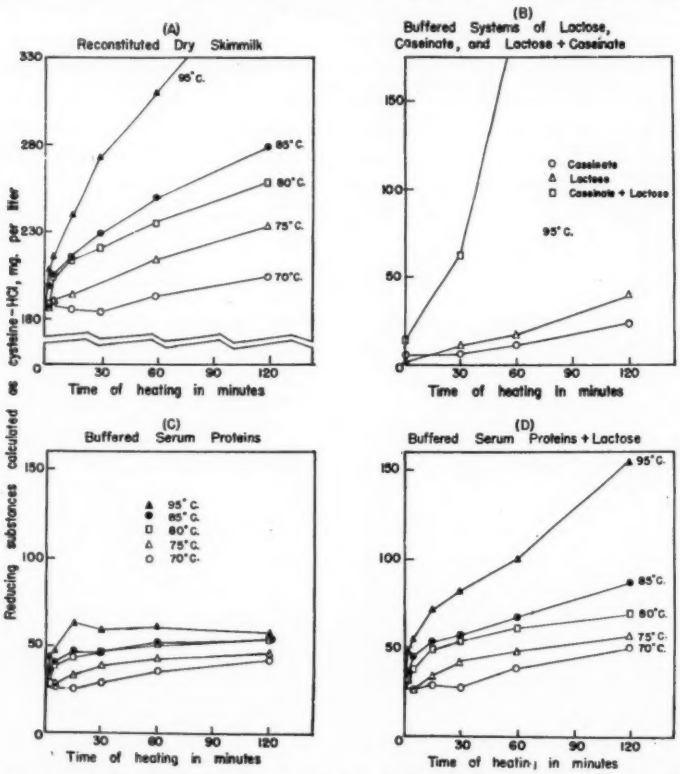

FIG. 3. The effect of heat treatment of skim milk and simplified systems on production of acid ferricyanide reducing substances. The points shown in these graphs are the average of at least two values.

For comparative purposes, skim milk was the first system studied (fig. 2A). It may be noted that no TDRS were produced during 120 minutes heating at 70° C., but they were produced slowly in skim milk during heating at 75° C. The maximum amounts of TDRS were produced following a brief (2 to 5 minutes) exposure at 85 and 95° C. The decline in reducing groups during long treatment at the higher temperatures may have some significance in processing milk where the object is that of obtaining maximum amounts of reducing substances.

The amount of TDRS obtained from the heat treatment of caseinate or caseinate plus lactose is practically negligible (fig. 2B).

The influence of heat treatment on TDRS in buffered serum proteins and buffered serum proteins plus lactose is illustrated in figures 2C and 2D, respectively. These two systems behave in a similar manner in their response to heat treatment when measured as TDRS, with the exception that the presence of lactose has a rather small but significant depressing effect on the amount of TDRS produced at each temperature. An incomplete reaction between the liberated sulfhydryl groups and the lactose or an inhibition by lactose of the denaturation of the protein (16) are alternate explanations for this difference. More TDRS are produced in serum protein systems than in an equivalent of the skim milk from which they were derived. This increased heat lability may be due either to slight denaturation of the protein during isolation or to the differences of environment in the milk and the simplified systems.

TABLE 2

Comparison of the nitroprusside and thiamin disulfide methods for detection of appearance and subsequent disappearance of heat-labile sulfur in milk

Time heated at 85° C.	Appearance		Disappearance		
				After storage[b]	
	Nitroprusside	TDRS (Cysteine-HCl)	Initial TDRS[a] (Cysteine-HCl)	Nitroprusside	TDRS (Cysteine-HCl)
(*sec.*)		(*mg./liter*)	(*mg./liter*)		(*mg./liter*)
0	nil	nil	14.4	++++	12.6
20	nil	0.2	14.4	++	3.3
30	±?	0.6	14.4	+	1.8
45	++	> 2.4	11.4	nil	nil
60	+++	> 2.4			

[a] Milk was preheated at 85° C. for 20 minutes.

[b] These samples were selected from among a number of comparisons. Storage was for one week at temperatures ranging from 4.4° C. to 37° C.

The data in figure 3 were obtained for comparison of AFRS and TDRS in the same systems. The capacity of milk to reduce ferricyanide recently has been considered in another paper (4). It should be pointed out that while the values of the TDRS discussed in the preceding paragraphs ranged from the equivalent of from 0.0 to 30 mg. of cysteine-HCl per liter, the AFRS on the same basis may exceed 500 mg. under similar conditions.

Only those systems containing both lactose and protein are characterized by the production of appreciable amounts of AFRS (fig. 3A, 3B and 3D) during heat treatment at 95° C. for 2 hours. The initial reducing capacity of the serum proteins is higher if measured by the acid ferricyanide method, but the two methods reveal a comparable increase in reducing capacity upon heating (compare fig. 2C and 3C).

It is difficult to prove exactly what groups in the protein are measured by thiamin disulfide. Some data have been obtained, however, which indicate that positive nitroprusside tests and the initial appearance of TDRS occur at about

the same level of heat treatment. Furthermore, upon storage of heated milk in air, the decrease in intensity and final disappearance of the reactive groups responsible for a positive nitroprusside test is paralleled by the level of TDRS. These data are shown in table 2. The nitroprusside and thiamin disulfide tests appear to detect the same groups.

Influence of oxygen tension on production of reducing groups by heat treatment. As mentioned earlier, it was found in preliminary trials that the presence of oxygen in the system caused a decrease in the amount of reducing substances produced during heating. Some further work on this point is reported in figure 4. The influence of deaeration before heat treatment at 85° C. on the production of AFRS and TDRS in reconstituted nonfat dry milk solids and fresh skim milk

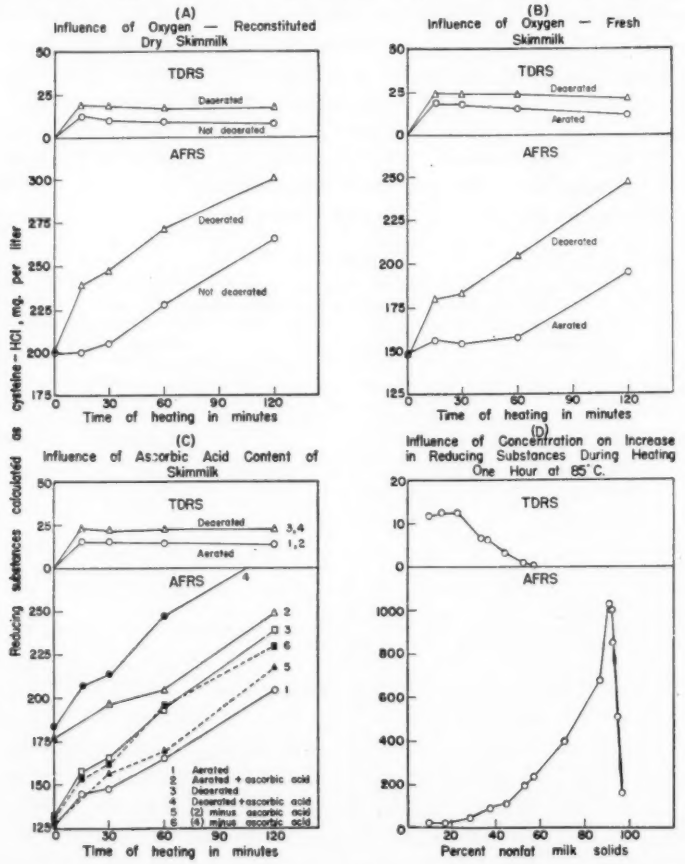

FIG. 4. The influence of oxygen, ascorbic acid and solids concentration on the reducing substances produced in nonfat milk solids during heat treatment at 85° C.

is shown in figures 4A and 4B, respectively. The presence of oxygen causes lower values for both AFRS and TDRS. Since the rates of production of AFRS become similar after about 30 minutes heating, regardless of the presence or absence of oxygen, it was considered possible that the initial lag in the production of reducing groups might be due to oxidation of the ascorbic acid. In an attempt to clarify this point, the data of figure 4C were obtained. The influence of oxygen and ascorbic acid on the TDRS during heating of skim milk is shown in the upper portion of figure 4C. Ascorbic acid was without effect on the TDRS, either in the presence or absence of oxygen. This result was not unexpected, since the thiamin disulfide reagent is not reduced by ascorbic acid. Any effect of ascorbic acid in this case would have to be indirect.

Curves 1 and 3 (fig. 4C) were obtained for the production of AFRS in samples of milk from which the ascorbic acid had been oxidized completely. Curve 1 represents an aerated and curve 3 a deaerated sample. Curves 2 and 4 are plots of similar data for the production of AFRS in the presence of 20 mg. of added ascorbic acid per liter. Curves 5 and 6 result from substracting the ascorbic acid from curves 2 and 4, respectively. Since the values for AFRS shown in curves 5 and 6 are similar to the corresponding curves 1 and 3 and also since curves 2 and 4 and 1 and 3 exhibit comparable effects of the presence of oxygen, it may be concluded that ascorbic acid is not a factor in determining the amount of AFRS produced during the heating of milk.

Influence of concentration of milk solids on production of reducing substances. In the course of this work, it was discovered that the concentration of the milk solids is an important factor in the effect of heat on the production of AFRS and TDRS in milk. Nonfat dry milk solids were used to prepare systems containing from 9.7 to 96.6 per cent solids for the determination of the production of AFRS during heating (fig. 4D). The rate of production of AFRS increases very rapidly with increase in the concentration of the milk within the range of 40 to 90 per cent solids, but decreases rapidly as the system approaches the moisture content of normal dry milk. This decrease in rate of production of AFRS with a decrease in the moisture content of dry milk recently has been reported by Coulter *et al.* (3).

The systems used for studying the effect of concentration on the production of thiamin disulfide reducing substances by heat were prepared by condensing fresh skim milk in an all-glass apparatus under reduced pressure. This was found to be necessary because attempts to prepare concentrated systems by dispersing nonfat dry milk solids in water resulted in a certain amount of the proteins becoming insoluble. The data obtained for the TDRS are indicated in the upper portion of figure 4D. The TDRS produced during heating remained fairly constant up to about 23 per cent solids and fell off rapidly to zero at 57 per cent solids.

DISCUSSION

A comparison has been made of acid ferricyanide and thiamin disulfide as oxidizing agents for heat-produced reducing substances in milk. The acid fer-

ricyanide reagent is the more powerful oxidant, and consequently reacts with other reducing groups in addition to those oxidized by the thiamin disulfide reagent. Since the nature of the AFRS has been discussed adequately in a recent paper by Crowe *et al.* (4), only comparisons with TDRS are necessary here.

Unheated milk or milk heated at temperatures up to 70° C. does not contain any substance capable of reducing thiamin disulfide, while unheated milk contains materials in addition to ascorbic acid which reduce acid ferricyanide. The TDRS are derived from heat denaturation of serum proteins. This is not surprising, since these proteins are known to contain most of the cystine-cysteine sulfur found in milk.

Although it is difficult to prove exactly what groups associated with heat treated milk serum proteins are measured by thiamin disulfide, the data presented in this paper show that this reagent probably is reduced by the same groups (undoubtedly sulfhydryls) that reduce the nitroprusside reagent used by several investigators in the past, notably Josephson and Doan (13). These workers have associated substances that reduce sodium nitroprusside with those producing a cooked flavor or those that provide the antioxygenic qualities to heated milk.

Although the presence of TDRS in dry whole milk appears to be associated with resistance to fat oxidation, these reducing substances did not decrease during storage of dry whole milk for 4 weeks in O_2 at 37° C. Even though Lea *et al.* (6, 15, 17) did not report any data concerning the stability of sulfhydryl-type reducing substances in aged dry whole milk, it is their contention that the initial presence of nitroprusside-reducing substances is concomitant with the resistance of the fat to oxidation in the stored milk.

The definitely higher reducing values obtained by heating milk in an atmosphere essentially free of oxygen agree with the statements of Greenbank and Wright (8) that deaeration of milk results in lower oxidation-reduction potential and greater protection of the resulting dry milk from oxidation. The results in figure 4C show that this effect is independent of the ascorbic acid content of the milk.

The fact that AFRS may be produced during spray drying and TDRS are not so produced can be accounted for by consideration of the effect of solids concentration on their production. TDRS are not produced on heating skim milk having a solids concentration higher than 60 per cent, whereas AFRS are produced at a maximum rate at about 90 per cent solids. Kitzes (14) has shown that the temperature of the drying particle is at about the wet-bulb temperature of the air during the early stages of drying but approaches that of the dry-bulb temperature of the air during the later stages. Thus the particle is subjected to high temperature only when its concentration is so high that TDRS are not produced.

SUMMARY AND CONCLUSIONS

Data have been presented indicating the source of thiamin disulfide reducing substances (TDRS) in heated milk and their relationship to acid ferricyanide

reducing substances (AFRS) as well as to the keeping quality of the dry whole milk. The influence of the oxygen tension and the concentration of the system on the production of TDRS and AFRS during the heat treatment have been studied.

The nitroprusside and thiamin disulfide methods for sulfhydryl groups in milk have been compared.

The following conclusions may be drawn from the results secured:

1. The substances that reduce thiamin disulfide are derived from the heat treatment of serum proteins and represent only a portion of the materials oxidized by ferricyanide.

2. The thiamin disulfide reducing substances appear to be associated with increased resistance to oxidation when present in dry whole milk but do not decrease in quantity as the milk fat is oxidized.

3. The presence of oxygen in milk being preheated depresses the amount of reducing substances produced.

4. The rate of production of thiamin disulfide reducing substances decreases with increase in the solids content of heated milk and becomes negligible at solids concentrations exceeding 60 per cent.

5. The rate of production of acid ferricyanide reducing substances in heated nonfat milk solids reaches a maximum at about 90 per cent solids.

6. The thiamin disulfide and nitroprusside reagents measure the same or parallel reducing systems in heated milk.

ACKNOWLEDGMENTS

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THE EFFECT OF VARYING PROPORTIONS OF EGG YOLK AND SODIUM CITRATE BUFFER IN BULL SEMEN DILUTERS UPON SPERM MOTILITY

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Since the first report of the influence of egg yolk upon bull sperm viability by Phillips (5), some form of egg yolk has been a major ingredient of a wide variety of diluters proposed for bull semen. Diluters containing egg yolk have produced satisfactory results with phosphate, citrate and sulfate buffers. Because of the ease of preparation and examination the citrate buffer has been the most popular in this country. Two separate types of investigation have made it of importance to examine diluters composed of egg yolk and citrate with a view to determining optimum concentrations for sperm viability.

An early report by Salisbury *et al.* (8) indicated satisfactory results with M/15 sodium citrate and egg yolk buffer, but weights listed in a later report (11) indicated that a M/7.5 solution had been prepared. The latter was used widely with apparently satisfactory results. The writer also had used M/15 citrate solutions satisfactorily. When the discrepancy was noted, a comparison was made between the freezing point depressions of fresh bull semen and the buffer solutions. As a result it was decided to use a concentration about mid-way between M/15 and M/7.5. Salisbury and Knodt (9) published a revised formula for citrate-egg yolk diluter using 3.6 per cent sodium citrate dihydrate. This revision later was explained (10) as being made to correct the osmotic pressure of the diluter to that of bull semen at the time of dilution. In the latter report Salisbury *et al.* (10) found that fresh normal bull semen had the same osmotic pressure as bovine blood and recommended a concentration of 2.9 per cent sodium citrate dihydrate to achieve this in the semen diluter prepared by heating. In view of apparently satisfactory results with such a wide variety of concentrations of sodium citrate, it seemed advisable to determine the limits of citrate concentration for satisfactory use in semen diluters.

Phillips' (6) original recommendation was for a mixture of one part of fresh egg yolk with one part of buffer solution. This pattern generally has been followed by all other investigators proposing the use of egg yolk in semen diluters. Mayer and Lasley (3, 4), in a study to determine the active fractions of egg yolk in protecting sperm against temperature-shock, found that egg yolk contained a harmful acetone-insoluble, alcohol-soluble fraction and a fraction insoluble in alcohol, acetone or ether, which was beneficial in minute amounts. Although they obtained a yield of 1.5 per cent of the latter material from egg yolk, it gave better protection than egg yolk at dilutions as high as 1 in 250 parts of buffer. These results suggested that the protective action of egg yolk might be obtained at lower concentrations than 50 per cent.

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EXPERIMENTAL

The effect of various concentrations of sodium citrate on bull semen was determined first, since any harmful effect of the buffer could mask the true effect of egg yolk in small concentrations. At the same time the protective action of large amounts of egg yolk also might protect the sperm against adverse concentrations of sodium citrate. A few preliminary trials showed that 25 per cent egg yolk gave practically the same effect on bull semen as 50 per cent in M/15 citrate diluter. Therefore the concentration of egg yolk was decreased to this point to allow for greater effect of the sodium citrate buffer solutions. Buffer solutions were prepared by mixing at room temperature 1, 2, 3, 4 and

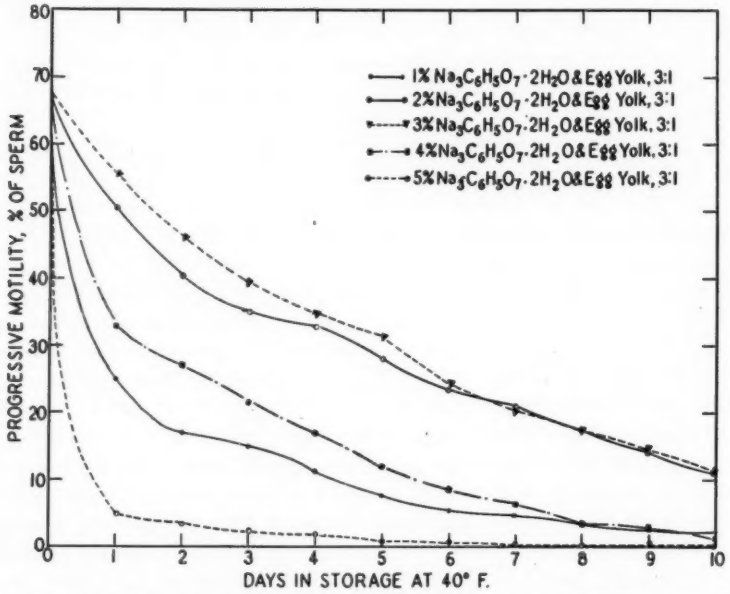

FIG. 1. Effect of various concentrations of sodium citrate in a yolk-buffer semen diluter upon motility of bull sperm (av. of 10 semen specimens).

5 g. of sodium citrate dihydrate per 100 ml. of water doubly distilled in glass. The semen diluters were prepared by mixing three parts of each buffer solution with one part of carefully separated egg yolk in 50 ml. graduated cylinders. Semen specimens from dairy bulls at the University of Tennessee were diluted identically with each of the five diluters before cooling below 28° C. The diluted samples were cooled slowly in insulated vials in a small electric refrigerator operating at 5° C. Estimations of the percentage of progressively motile sperm were made at daily intervals for 10 days. The average results from ten different semen specimens diluted at the rate of one part of semen to ten or twenty parts of each diluter are shown graphically in figure 1. It is apparent that the diluters made from 2 or 3 per cent sodium citrate dihydrate allowed better

TABLE 1

Freezing points of various sodium citrate buffer solutions and semen diluters prepared from three parts buffer and one part egg yolk

Concentration of $Na_3C_6H_5O_7 \cdot 2H_2O$	Freezing point	
	Buffer	Diluter
(%)	(°C.)	(°C.)
1	−0.19	−0.25
2	−0.38	−0.40
3	−0.57	−0.56
4	−0.72	−0.70
5	−0.88	−0.85

motility than the others, and in comparison with these the 4 per cent citrate diluter seemed unsatisfactory. The 5 per cent citrate diluter had an immediate adverse effect on sperm motility. The 1 per cent citrate diluter was tolerated better than the 5 per cent, but, in addition to failing to maintain satisfactory motility, it caused a high proportion of coiled-tail sperm which could move only backwards or in circles.

In view of the importance of osmotic pressure upon cell metabolism, the freezing points of the buffer solutions and the diluters prepared in this experiment were determined. These data are presented in table 1. For comparison, the freezing point of a composite semen sample from three bulls was determined.

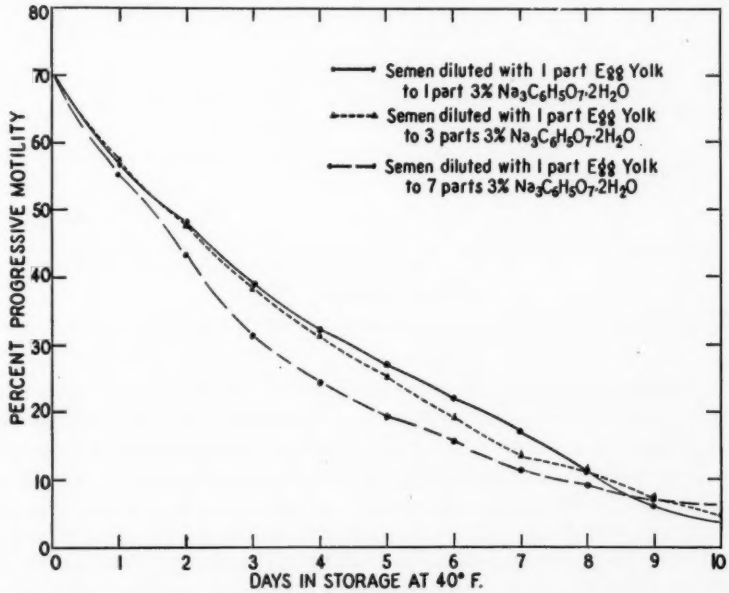

FIG. 2. Comparison of effect of 50, 25, and 12.5% egg yolk in semen diluter upon motility of bull sperm (av. of 11 semen specimens).

The freshly collected semen was cooled in ice water and the freezing point was determined within 1 hour. An average value of $-0.56°$ C. was found. The diluter prepared with 3 per cent citrate buffer was found to have the same freezing point as fresh bull semen. This solution also resulted in the best average motility. With two of the semen samples the diluter made with 2 per cent citrate resulted in motility equal to that in the 3 per cent citrate diluter. In no instance was the 4 per cent diluter equal to the 3 per cent diluter. Apparently bovine sperm are more sensitive to hypertonic solutions of sodium citrate than to hypotonic solutions. The freezing point of four egg yolks averaged $-0.57°$ C. It would be expected that the egg yolk would not affect significantly the tonicity of a diluter made with isotonic buffer solution.

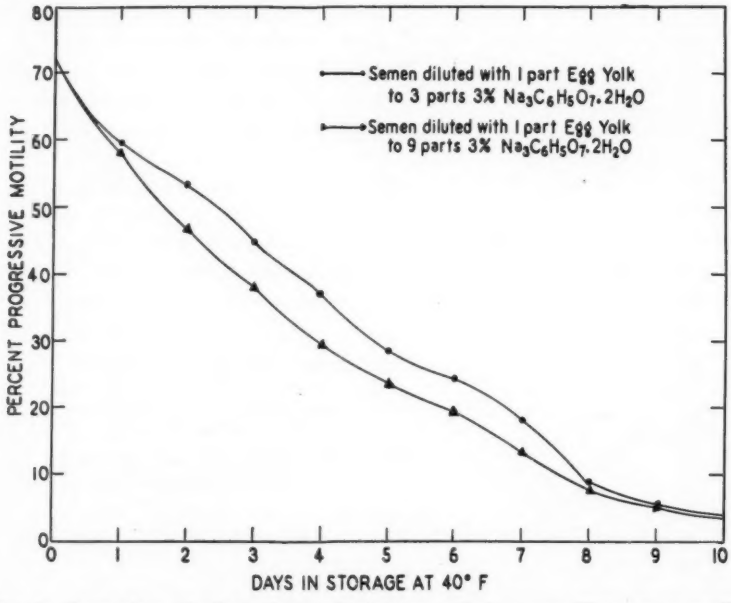

FIG. 3. Comparison of effect of 25 and 10% egg yolk in semen diluter upon motility of bull sperm (av. of 11 semen specimens).

In view of the results from the first part of the experiment a buffer solution of 3 per cent sodium citrate dihydrate was adopted for the comparisons of egg yolk concentration. All samples of semen used in this phase of the experiment were not treated with each concentration, but series of trials were made in which three or four different concentrations of egg yolk were compared. The semen generally was diluted at the rate of one part semen to ten parts diluter. In the first trial 11 semen specimens were diluted identically with diluters made with 50 per cent, 25 per cent and 12.5 per cent egg yolk, respectively. The average daily progressive motility of semen stored at 5° C. noted in this trial is shown graphically in figure 2. The 25 per cent egg yolk diluter resulted in

practically the same motility as the 50 per cent diluter. The 12.5 per cent egg yolk gave very good motility for the first 2 days of storage but was definitely inferior to the other two diluters.

In the second trial comparisons were made between 25 per cent egg yolk and 10 per cent egg yolk and citrate diluter. Charts of the average daily progressive motility ratings of 11 semen specimens used in this trial are shown in figure 3. The 10 per cent egg yolk, although it was not as good as the 25 per cent diluter, maintained surprisingly good sperm motility, quite comparable to that observed with the 12.5 per cent diluter.

A third trial was made to attempt to locate the limit of egg yolk concentration which would be effective in maintaining motility. In this trial comparisons were

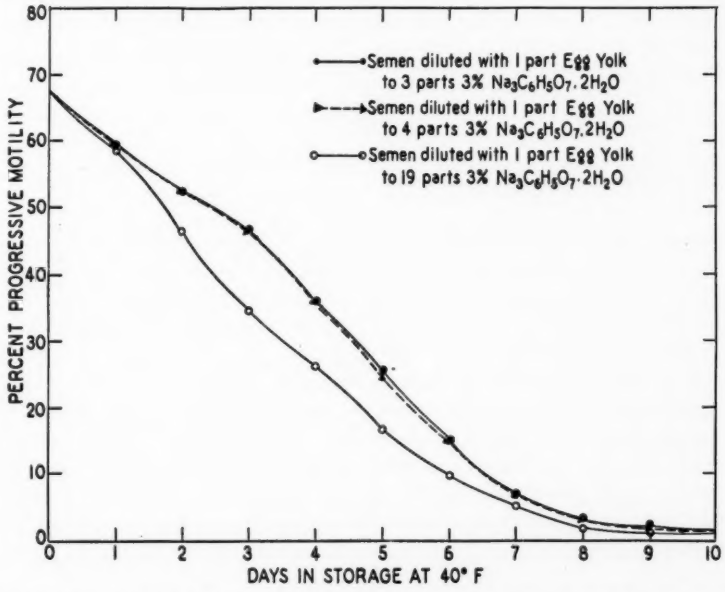

FIG. 4. Comparison of effect of 25, 20, and 5% egg yolk in semen diluter upon motility of bull sperm (av. of 11 semen specimens).

made between 25 per cent, 20 per cent, and 5 per cent egg yolk diluters. The average daily progressive motility ratings found from the 11 semen specimens used in this trial are plotted in figure 4. Identical effects were observed with 20 and 25 per cent egg yolk in the diluter. When only 5 per cent egg yolk was used, a greater difference was observed after the first day of storage than had been observed between the 10 or 12.5 per cent egg yolk and 25 per cent egg yolk diluters. It was clear, however, that even this very low concentration of egg yolk provided some beneficial effect. Two samples of semen diluted with only the buffer solution declined to 5 per cent progressive motility in 96 hours while the same semen diluted with only 5 per cent egg yolk in the diluter had 20 per cent progressive motility at 96 hours.

TABLE 2

Effect of varying proportions of egg yolk and 3 per cent sodium citrate buffer upon the resistance of bull sperm to cold shock

Diluter	Live sperm		Sperm killed
	Before shock	After shock	
	(%)	(%)	(%)
Series I (av. of 3 samples uncooled semen)			
No diluter	81	4	77
1:1, yolk:buffer	85	55	30
1:3, yolk:buffer	78	46	32
1:7, yolk:buffer	80	46	34
Series II (av. of 2 samples uncooled semen)			
No diluter	69	2	67
3% citrate buffer	74	1	73
1:3, yolk:buffer	73	35	38
1:9, yolk:buffer	74	33	41
Series III (av. of 7 samples cooled and stored semen)			
1:3, yolk:buffer	78	69	9
1:4, yolk:buffer	80	67	13
1:19, yolk: buffer	73	54	19

Since the protective action of egg yolk against temperature shock of sperm is a very important factor in the use of semen diluters, a study was made of the effect of the various concentrations of egg yolk in preventing death of sperm subjected to rapid cooling. Smears for the determination of live and dead sperm were made, by a staining technic of Lasley *et al.* (2), before and after cold shocking by placing test tubes of semen at 20° C. in a brine bath at 0 to −1° C. for 10 minutes. Three series of semen samples prepared for the storage studies reported above were treated in this manner. The cold shock treatment was given two series of samples within a few minutes after collection of the semen and before cooling had proceeded below room temperature. The third series was studied after the samples had been cooled gradually and kept in storage at 5° C. for at least 24 hours. These comparisons were presented in table 2.

TABLE 3

Effect of varying proportions of egg yolk and citrate buffer upon the resistance of bull sperm to cold shock before and after storage at 5° C.

Diluter	Before cooling			After 72 hr. at 5° C.		
	Live sperm		Sperm Killed	Live sperm		Sperm Killed
	Before Shock	After Shock		Before Shock	After Shock	
	(%)	(%)	(%)	(%)	(%)	(%)
No diluter	57	15	42	38	12	26
3% citrate buffer	65	18	47	63	33	30
1:4, yolk:citrate	71	40	31	63	50	13
1:9, yolk:citrate	70	41	29	60	48	12
1:19, yolk:citrate	68	43	25	62	43	19

It is evident from these results that diluter containing 12.5 or 10 per cent egg yolk was as effective in protecting against cold shock as was diluter containing 25 or 50 per cent egg yolk. The trial with stored semen indicated that 5 per cent egg yolk was inferior to 20 or 25 per cent in protective action, but that all concentrations exerted greater protective action than observed in uncooled semen. This effect was checked in a fourth series of three semen samples from different bulls with which the protective action of egg yolk was determined before cooling and after 72 hours storage at 5° C. The average results of this trial are presented in table 3.

These data indicate that 5 per cent egg yolk was practically as effective in protecting sperm against cold shock as were concentrations of 10 and 20 per cent. The increased resistance of sperm to cold shock after storage was noted as before, but it occurred in samples not diluted with egg yolk as well as those containing egg yolk. Some change may occur in stored sperm which affects their staining properties in addition to their acquisition of the egg yolk protective factor.

DISCUSSION

These experiments have demonstrated that the concentration of sodium citrate in the semen diluter may influence the viability of sperm. The optimum concentration was found to be that which gave a freezing point nearest that of freshly collected semen. This indicates that the influence of osmotic pressure may be important in preparing semen diluters. This is in agreement with the report of Salisbury *et al.* (10). The observation that hypotonic solutions were less harmful than hypertonic may be explained by the fact that as semen ages, lactic acid increases, causing an increase in the osmotic pressure which could be counteracted by a hypotonic solution, but only aggravates an incompatible condition in a hypertonic solution. It may be shown by later, more definitive experiments that some concentration of sodium citrate between 2.5 and 3 per cent is superior to 3 per cent.

The experiments with varying concentrations of egg yolk indicate that satisfactory results may be expected with much less than 50 per cent yolk in the diluter. The point of critical concentration was not determined definitely. From viability studies it would appear to be somewhere between 20 and 12.5 per cent. The former gave the same motility as with 50 per cent yolk, while the latter was lower in motility after the first 2 days of storage. Whether or not differences would appear in fertility cannot be decided without extensive breeding trials. Bovine semen used at the University of Tennessee has been diluted with 3 per cent sodium citrate buffer and egg yolk, three or four parts to one, routinely for over 12 months. This semen has been used for inseminating up to 3 days of age with excellent results.

Reducing the egg yolk concentration to 10 per cent had no apparent effect upon the protective action during cooling or cold shock. Since motility was not maintained as well with 10 per cent yolk as with 20 to 50 per cent, the lack of sufficient protective factor may be considered of secondary importance in mixing semen diluters with egg yolk. Factors necessary for the maintenance of the

highest motility other than protective factor must be present in egg yolk. One of these may be viscosity. Knoop (1) found that gelatin increased livability of sperm and Phillips and Spitzer (7) reported beneficial effects on sperm livability of certain gums. These substances increase the viscosity of diluted semen and reduce the motility during storage. Perhaps a certain amount of egg yolk (20 to 25 per cent) provides a similar condition.

Reducing the amount of egg yolk used in preparing semen diluter not only makes the preparation of the diluter more economical and less laborious, but results in a relatively clear field under the microscope in which estimation of semen motility is easier and more precise than when large concentrations of egg yolk are used.

SUMMARY

An investigation has been made of the egg yolk-sodium citrate buffer diluter for bull semen with the view of establishing optimum concentrations. It was found that 3 per cent sodium citrate dihydrate was superior to other concentrations tried, and this solution was nearly isotonic with fresh bull semen. Reducing egg yolk concentration to 20 per cent gave as good motility and livability as the commonly used 50 per cent. When egg yolk was reduced to 10 per cent the cold shock protective factor was still fully effective but motility was impaired slightly.

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OXYGEN DAMAGE TO BULL SPERMATOZOA AND ITS PREVENTION BY CATALASE[1]

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In the practice of diluting and storing bull semen for use in artificial breeding it has been accepted generally that the spermatozoa remained motile longer if stored with a minimum of air space above the semen and subjected to a minimum of mixing.

MacLeod (5) showed in 1941 that high oxygen tensions were harmful to human spermatozoa. On the basis of the first experiment reported here, which was completed in 1944, and other evidence, Salisbury (10) reported that bull spermatozoa in low concentrations were harmed by oxygen.

MacLeod (6) found that adding catalase or peroxidase to human semen would prevent the harmful effects of the oxygen. This suggested that the deleterious effect of oxygen on spermatozoa was due in part to hydrogen peroxide. Hydrogen peroxide has been shown to be toxic to human (6) and bull spermatozoa (3).

Recent investigations by Tosic and Walton (15, 16) and by Tosic (14) have shown that bull spermatozoa stored under aerobic conditions produced hydrogen peroxide. They have shown that an oxidative deamination of L-tryptophane, L-tryosine, or L-phenylalanine by the spermatozoa was responsible for the production of hydrogen peroxide and this in turn resulted in an inhibition of spermatozoan motility and respiration. This inhibition was reversed when catalase was added.

These reports suggest that oxygen damage to diluted bull semen may occur in the routine handling of bull semen for artificial insemination, and that the procedures carried out involving shaking the semen may increase these effects through increased aeration and exposure to the oxygen of the air. Since the experiments reported in this paper were completed, Prince and Almquist (9) have shown that agitation of diluted bull semen was more harmful in partially filled tubes than in full tubes.

The experiments reported here were designed to determine (a) the effect of storing diluted bull semen under nitrogen, oxygen and air on the activity of the spermatozoa, (b) whether deleterious effects from oxygen, if present, could be prevented by catalase, (c) whether oxygen damage to spermatozoa is increased by gentle mixing of diluted semen under conditions of routine artificial breeding and, (d) whether or not catalase would improve livability of spermatozoa under routine storage conditions.

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EXPERIMENTAL PROCEDURE

The semen samples used for these experiments were collected from the bulls of the New York Artificial Breeders' Cooperative and examined by the procedures described earlier (17). Each of the ten ejaculates used in testing the effects of incubation and storage under air, oxygen and nitrogen was diluted at the rate of one part of semen to four parts of the yolk citrate diluent (13). One ml. aliquots of the diluted semen then were measured into three series of four test tubes each. Each series consisted of one small 10×75 mm. tube and three larger 18×150 mm. tubes. The small test tube and one large test tube of each series then were stoppered; these were considered as being "under air." The other two large tubes of each series were closed with two-holed rubber stoppers equipped with one long and one short glass tube. One of these large test tubes of each series then was oxygenated for 0.5 minute by allowing a slow stream of oxygen to blow on the surface of the diluted semen while the semen was agitated gently. Following oxygenation the tube was closed immediately by means of rubber tubes and pinch clamps. A similar operation was carried out with the remaining tube of each series using nitrogen. One series of the four variously treated tubes then was incubated for 1 hour at 46.5° C.; the other two series were cooled slowly and stored at 5° C.

Aliquots of the diluted semen were taken prior to and at the end of incubation for 1 hour and after low-temperature storage of 4 and 10 days for analyses to determine the change in utilizable sugars and the accumulation of lactic acid. At the time when these analyses were carried out, the change in total reducing sugars was thought to be essentially a glucose loss; however, more recent findings by Mann (7) have shown the principal sugar in semen to be fructose. The changes in reducing sugar, called sugar loss, and lactic acid reported in this paper were obtained by difference between analyses prior to and those after incubation and after low-temperature storage. Slight modifications of the micro blood sugar method of Horvath and Knehr (2) and the lactic acid method of Barker and Summerson (1) were used for the analyses.

The percentage and rate of spermatozoan motility were estimated following both incubation and low-temperature storage.

In testing the effectiveness of catalase in preventing the harmful action of oxygen on spermatozoa, ten ejaculates of bull semen at dilution rates of one part of semen to four or 100 parts of egg yolk-citrate diluent were used (11). One ml. aliquots of each then were measured into three series of four 18×150 mm. test tubes. To two of the tubes of each series was added 0.1 ml. M/15 phosphate buffer containing approximately 0.2 to 0.4 of a unit of beef liver catalase; the other two tubes of each series received 0.1 ml. additions of the phosphate buffer. One tube without and one tube with added catalase of each series then were stoppered and considered as being "under air". The remaining two tubes of each series were oxygenated as described above.

One series of each dilution rate was incubated for 1 hour at 46.5° C. and the other two were cooled and stored at 5° C. At the termination of incubation and after 4 days and 10 days of low-temperature storage, the samples were opened

and the percentage and rate of spermatozoan motility estimated by microscopic examination.

To test the effects of gentle mixing on spermatozoan livability during low-temperature storage and to determine simultaneously whether catalase would improve livability, ten samples of semen diluted at 1:4 and 1:100 with the yolk-citrate diluent were used. Four 15×125 mm. test tubes, two with 0.2 ml. M/15 phosphate buffer added and two with 0.2 ml. of the phosphate buffer containing 0.4 to 0.8 of a unit of catalase added, were prepared for each ejaculate and each dilution rate. Two ml. of diluted semen were added to each tube, mixed and the tubes stoppered and cooled to 5° C.

During storage at 5° C., one tube with and one tube without added catalase of each dilution and each ejaculate was mixed gently at approximately hourly intervals. Each gentle mixing consisted of inverting the tubes ten times very slowly during the course of about 0.5 minute and then removing the stoppers and replacing them after another 0.5 minute. This operation was carried out on the average of seven times a day for the first 5 days and then discontinued. This was considered to duplicate to a large extent the conditions of mixing the semen at each breeding as carried out by inseminators in the field.

The remaining tubes, one with and one without added catalase at each dilution, were used as controls and were mixed only once a day at the time of examination for motility. Motility examinations were made daily for 10 days on all samples.

RESULTS

Spermatozoan activity during incubation and storage under air, oxygen and nitrogen. The mean initial semen characteristics of the ten ejaculates used in this study were as follows: sperm count, 988,000 per mm.^{3}; initial motility, 63 per cent; rate of motility, 3.65 (maximum = 4); and methylene blue reduction time, 10.5 minutes. The average quality of these ejaculates was slightly below that normally required for actual breeding by the New York Artificial Breeders' Cooperative.

The results of this study are summarized in table 1, where the means of the observations on the ten ejaculates during incubation and storage are presented. This table is arranged so that the results for the four tubes of a series fall into the order of decreasing quantity of oxygen in the tubes (O_2, large tube with a large volume of air, small tube with comparatively less air, and N_2).

From table 1 it can be seen that, with the exception of the small tube and large tube at low-temperature storage after 4 days, each decrease in the relative amount of oxygen present resulted in a slower decline in the percentage and rate of spermatozoan motility. Although the methods used did not give an absolute measure of the oxygen present, the results show that reducing the relative amount of oxygen sealed in the storage tube produced a notable increase in livability during incubation and low-temperature storage. An analysis of variance of the motility observations showed a highly significant difference between treatments (air, O_2, and N_2) for both percentage and rate of motility.

Under oxygen, less sugar disappeared during incubation and storage for 4 days than was lost from the other treatments. However, after storage for 10 days there were no significant differences in sugar losses between any of the treatments. This was the same as was found earlier when sugar disappeared after storage for 10 days independent of semen quality (12).

Lactic acid gain, on the other hand, paralleled the increases in motility with the exception of the nitrogen treatment. In every case less lactic acid accumulated under nitrogen than under air, but more than under oxygen. Under oxygen less lactic acid was present at 10 days than was present at 4 days. This suggests that the lactic acid was oxidized.

The figures for the percentage recovery of sugar lost as lactic acid indicate that during storage for 4 days lactic acid was produced from some source other

TABLE 1

Mean per cent motility, rate of motility, sugar loss, lactic acid gain and per cent recovery of sugar lost as lactic acid in 10 ejaculates of bull semen after incubation and low temperature storage under air, oxygen, and nitrogen

After incubation for 1 hr. at 46.5° C.				After storage at 5° C.							
				4 days				10 days			
O_2	Large tube[a]	Small tube[a]	N_2	O_2	Large tube[a]	Small tube[a]	N_2	O_2	Large tube[a]	Small tube[a]	N_2
Motility (%)											
1	21	30	40	34	55	55	63	10	32	34	39
Rate of motility (max. = 4.0)											
0.1	1.2	1.6	2.2	1.7	3.1	3.1	3.3	0.5	1.4	1.6	1.8
Sugar loss (mg./100 ml.)											
31	51	62	60	30	58	60	47	84	89	78	88
Lactic acid gain (mg./100 ml.)											
24	45	50	49	41	53	59	48	31	65	68	52
Recovery of sugar lost as lactic acid (%)											
77	88	81	82	137	91	98	102	37	73	87	59

[a] Under air.

than the sugar, since the sugar loss was not enough to account for the accumulated lactic acid. From the percentage recovery of sugar lost as lactic acid under nitrogen during storage for 10 days it appears that either lactic acid is utilized by the spermatozoa, thus reducing the total accumulation, or that some of the sugar lost is broken down to a product other than lactic acid. Lardy and Phillips (4) have indicated that spermatozoa can utilize lactic acid for the maintenance of motility.

Prevention of the ill effects of oxygen on spermatozoa by the use of catalase. The results in the study of ten ejaculates of semen stored under oxygen and under air, with and without added catalase, are presented in table 2. From these data it can be seen that oxygen again was harmful to spermatozoan motility, thus confirming the findings of the first experiment. The presence of catalase in semen diluted either at the low or high rate and incubated or stored under oxygen was effective in maintaining a higher percentage of motile spermatozoa and at a higher

TABLE 2

Mean percentage and rate of spermatozoan motility of 10 ejaculates of bull semen at two dilution rates after incubation and storage under oxygen and air with and without added catalase. (Mean initial motility 63%; rate, 3.2)

After incubation for 1 hr. at 46.5° C.				After storage at 5° C.							
				4 days				10 days			
O_2	O_2 + Cat.	Air	Air + Cat.	O_2	O_2 + Cat.	Air	Air + Cat.	O_2	O_2 + Cat.	Air	Air + Cat.
Motility (%)				1:4 Dilution							
4	42	41	44	14	38	40	43	8	17	8	22
Rate of motility[a]											
0.3	2.2	2.4	2.5	0.6	1.8	2.0	2.2	0.4	0.8	0.4	1.0
Motility (%)				1:100 Dilution							
1	20	14	19	7	24	30	37	1	3	3	10
Rate of motility[a]											
0.1	1.4	1.2	1.4	0.4	1.3	1.5	1.7	0.1	0.1	0.2	0.5

[a] Maximum = 4.

rate of motility. The results show that those samples stored under oxygen in the presence of catalase maintained their rate and percentage of motile spermatozoa as well as, or better than, those under air during incubation and after storage for 10 days at 5° C. However, after 4 days at 5° C., motility was slightly better in the samples stored under air than under oxygen with added catalase.

Without exception the samples under air to which catalase had been added

TABLE 3

Comparison of the effects of gentle mixing versus no mixing on the mean per cent of motile sperm in 10 ejaculates of bull semen at two dilution rates stored with and without added catalase. (Mean initial motility, 63%)

Sample	Days Storage at 5° C.									
	1	2	3	4	5	6	7	8	9	10
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
					1:4 Dilution					
Non-mixed[a]										
Control	56	53	47	42	34	24	16	10	6	4
+ Cat.	56	53	48	46	42	36	32	24	15	12
Mixed[b]										
Control	55	50	37	31	23	16	9	6	4	2
+ Cat.	56	52	47	42	38	33	28	17	12	9
					1:100 Dilution					
Non-mixed										
Control	54	45	37	26	17	8	4	4	2	1
+ Cat.	54	45	38	35	26	20	17	12	7	7
Mixed										
Control	54	41	26	18	10	5	2	2	2	1
+ Cat.	54	44	36	28	20	15	10	8	5	5

[a] Non-mixed = no mixing except at the daily observations.

[b] Mixed = gently mixed by inverting, this operation repeated on the average of 7 times a day for the first 5 days.

were higher in per cent and rate of spermatozoan motility than the samples under air but without the added catalase. After storage for 4 days at 5° C. the samples diluted at the rate of 1 to 100 averaged 7 per cent more motile sperm than when no catalase was added. This suggests that the addition of catalase to diluted semen used in routine breeding in the field might lengthen its useful life.

From the protective action of catalase found in these studies it seems reasonable to postulate that at least a part of the deleterious effects of high oxygen tension on spermatozoan motility is caused by increased H_2O_2 production. The increased production of H_2O_2 in turn results in the more rapid death of the spermatozoa.

The effects of gentle mixing on spermatozoan motility in diluted semen and the beneficial action of catalase. The effects of gentle mixing on the maintenance of spermatozoan motility in the presence and absence of added catalase are shown in table 3. The data on the rate of spermatozoan motility closely paralleled that for percentage motility and therefore are not presented here. Gentle shaking reduced considerably the percentage of spermatozoan motility in the control samples at both the 1:4 and the 1:100 dilution rates. However, if the mixed samples contained added catalase, motility was maintained better than in non-mixed samples without added catalase. In fact, the presence of added catalase slowed the decline in the percentage of motile spermatozoa in both the mixed and non-mixed samples. Livability was greatest in the non-mixed samples with added catalase. Next were the mixed samples with added catalase, followed by the non-mixed samples without added catalase. The maintenance of motility was poorest in the mixed samples without added catalase. This latter group was nearest to the conditions normally carried out in the field in routine artificial breeding.

Since catalase was effective in preventing the ill-effects of gentle shaking, it seems reasonable to conclude that the effect of shaking was due primarily to the increased production of H_2O_2 by the spermatozoa, and not to mechanical injury. Mercier (8) found that increased gentle shaking did not increase the number of tailless heads or abnormal forms of spermatozoa found in slides prepared for staining.

The beneficial action of catalase, when samples received minimum mixing necessitated by the daily examinations, indicates that the spermatozoa were producing H_2O_2 even when subjected to little extra aeration.

DISCUSSION

Although the oxygen concentrations were not controlled carefully, the results of the first experiment indicated that near-anaerobic conditions were desirable for the maintenance of bovine spermatozoan motility. This is similar to the findings of MacLeod (5), who has shown that the higher the oxygen tension the quicker human spermatozoa lose their motility. Thus for artificial breeding purposes, diluted bull semen should be stored in test tubes with a minimum of air space left above the semen. Storage tubes of various sizes should be used to ship the semen in order to have each tube as nearly full as possible in order to cut down on the volume of air and minimize the deleterious effects due to oxygen.

Since the present-day methods of shipping and handling bull semen for use in artificial insemination involve unavoidable gentle-mixing procedures, the results of the experiments presented here suggest that adding catalase to semen diluted with the egg yolk-citrate would prove beneficial. The production of hydrogen peroxide undoubtedly is increased by mixing procedures. Added catalase would aid in the rapid destruction of the increased quantities of hydrogen peroxide produced and thereby maintain a less toxic environment for the spermatozoa, thus promoting longer livability. An experiment to test the effects of added catalase on conception results in routine artificial breeding in the field is underway and will be reported at a later date.

SUMMARY

1. Bovine spermatozoan activity was studied in the egg yolk-citrate diluent during incubation at 46.5° C. and storage at 5° C. under air, oxygen and nitrogen, under air and oxygen with and without added catalase, and during storage when subjected to minimum and routine mixing procedures with and without added catalase.

2. Oxygen produced deleterious effects which were manifested by decreased motility and livability of the spermatozoa and a reduction in their ability to convert sugar to lactic acid.

3. Oxygen damage to spermatozoa was largely eliminated when catalase was added to the diluted semen.

4. Gentle mixing shortened the life of stored bull spermatozoa in the yolk-citrate diluent, but the presence of added catalase in mixed samples obliterated the harmful effects of mixing.

5. The effectiveness of catalase in preventing the harmful action of oxygen and shaking on bull spermatozoa in the egg yolk-citrate diluent led to the conclusion that increased aeration and higher oxygen tensions speed the production of hydrogen peroxide by the spermatozoa and this in turn produces a toxic environment, shortening the life of the spermatozoa.

6. The results suggest that a minimum of air space be left above the diluted semen that is stored and shipped in routine artificial breeding and that semen samples should be subjected to a minimum of mixing.

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USE OF DEHYDRATED BANANA MEAL IN THE RATIONS OF DAIRY CALVES[1, 2, 3]

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Dehydrated banana meal, because of its high pectin content, palatability and the proven value of bananas in the treatment of diarrhea in infant and adult humans (1, 2, 3, 4, 5) appeared to be of possible value in the rations of dairy calves. Analysis of dehydrated banana meal prepared from whole bananas indicated the following composition: crude protein, 4.70 per cent; ether extract, 3.10 per cent; nitrogen free extract, 76.10 per cent; crude fiber, 4.30 per cent; pectins and tannins, 9.15 per cent. A review of available literature revealed no reports relative to the use of this product in animal feeds.

The objective of the trials presented in this report was to determine the value of dehydrated banana meal for growth and for the prevention of scours in dairy calves.

EXPERIMENTAL PROCEDURE

Six groups of 10 Holstein male calves each were used in the trials presented in this report. The calves were obtained in eastern Pennsylvania from private and state-owned herds. At the beginning of the trials, the calves were divided into six comparable groups on the basis of body weight, height at withers, circumference of the chest, and age.

The calves were housed in an artificially heated and ventilated barn. They were maintained in individual pens provided with a water bowl, grain box, hay rack and salt block. The calves were distributed throughout the barn to avoid as much as possible any positional effects. Measurements of body weight, height at withers and chest circumference were taken each Tuesday at 9:00 a.m. and the calves always were weighed in the same order. Daily observations were made of the condition of the feces of each calf. All observations were made by the same person to minimize as much as possible any variation. Six categories were set up relative to the general character of the feces as follows: W = white scours; WB = white scours with blood; N = normal; N1 = loose; N2 = loose +; N3 = very loose; N4 = watery.

All calves were given one bolus (4 g.) of sulfathalidine at the time of each of the first four feedings after arrival at the experimental barn. If scours oc-

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[3] Authorized for publication on November 19, 1948, as paper No. 1489 in the journal series of the Pennsylvania Agricultural Experiment Station.

curred and lasted more than 24 hours during the trial, the calf again received the above treatment.

The calves were fed whole milk, which averaged about 3.7 per cent butterfat, from the College research herd. This was fed twice a day according to the following schedule:

1–4 weeks	10 lb. per day
5 "	8 " " "
6 "	6 " " "
7 "	4 " " "

In addition to milk, the calves were fed their respective group grain mixture *ad libitum* up to 6 lb. per day. All of the grain mixtures as presented in table 1 contained approximately 17.35 per cent of digestible protein. Group I served

TABLE 1

Rations used

Ingredients	Group I	Group II	Group III	Group IV
		(*lb.*)		
Banana meal	0	100	400	800
Ground yellow corn	600	500	200	125
Rolled oats	500	488	450	50
Wheat bran	200	200	200	200
Soybean meal	250	262	300	375
Dried skim milk[a]	400	400	400	400
Salt	20	20	20	20
Ground limestone	10	10	10	10
Steamed bonemeal	10	10	10	10
Vit. A oil (3300 USP units/g.)	8	8	8	8
Irradiated yeast	2	2	2	2
Estimated dig. protein (%)	17.33	17.30	17.38	17.43

[a] At the end of 4 months 300 lb. of soybean meal were substituted for 300 lb. of skim milk powder.

as a control group and received no banana meal. Group II received a ration containing 5 per cent banana meal; group III, 20 per cent; and group IV, 40 per cent. Groups V and VI received the basal ration plus 2 and 4 ounces of banana meal, respectively, twice daily, mixed with the milk fed. The whole milk was fed to these groups at the same rate as to the other four groups.

During the first 4 weeks, orchard grass-alfalfa hay of good quality was fed *ad libitum*. After the first 4 weeks, good quality alfalfa hay was fed *ad libitum*. The calves in groups I, II, III and IV were on the trial 24 weeks. The calves in groups V and VI were on trial for 8 weeks, 1 week in addition to the milk feeding period.

RESULTS

Growth. The relative rates of growth of the various groups of calves were recorded in terms of pounds of body weight, inches of chest circumference and height at withers in inches. The summarized data at 8 and 24 weeks are presented in table 2. The most rapid growth during the first 8 weeks was made by

TABLE 2

Rates of growth of calves[a]

	Group I	Group II	Group III	Group IV	Group V	Group VI
			Body weight (lb.)			
Initial	102.0	100.7	99.7	99.0	103.2	111.9
8 weeks	174.3	175.6	168.9	181.4	155.4	186.6
24 weeks	432.6	445.6	420.8	428.6	.....	.....
			Height at withers (in.)			
Initial	29.7	29.5	29.8	30.0	29.9	29.8
8 weeks	33.0	33.0	33.0	33.7	32.6	33.5
24 weeks	41.7	41.2	41.2	41.2	.....	.....
			Chest circumference (in.)			
Initial	31.7	31.5	32.0	31.5	31.2	33.3
8 weeks	37.6	37.8	37.1	37.9	36.4	38.2
24 weeks	51.3	51.6	51.0	51.8	.....	.....

[a] Each datum represents a mean of the observations on all calves in the given group at the given time.

group IV. This group gained an average of 1.47 lb. daily as compared to 1.29, 1.34, 1.24, 0.93 and 1.33 lb. for groups I, II, III, V and VI, respectively. The lowest rate of growth, as indicated by body weight gain, was in group V, which was the group that received 2 ounces of banana meal mixed with each of two daily milk feedings. Statistical treatment of the data by analysis of variance indicated that there was no significant difference among groups in body

TABLE 3

Incidence of abnormal feces and use of sulfathalidine treatments during first 8 weeks (10 calves per group)

	Group I	Group II	Group III	Group IV	Group V	Group VI
No. of cases[a,b]						
W	0	3	2	1	8	5
WB	0	0	0	0	0	3
N1	14	15	20	22	12	8
N2	2	3	5	2	4	7
N3	0	0	2	1	0	3
N4	4	0	2	2	0	5
Total	20	21	31	27	24	31
No. calves having abnormal feces[a]	7	9	9	10	9	8
Av. duration (days)	1.5	1.4	1.7	1.1	1.1	2.7
No. receiving one treatment sulfathalidine[a]	1	4	2	2	5	4
No. receiving 2 or more sulfathalidine treatments[a]	1	0	4	1	2	1

[a] After initial treatment.

[b] W = white scours
WB = white scours with blood
N1 = loose
N2 = loose +
N3 = very loose
N4 = watery

weight after being on the trial 8 weeks. There was little variation in height at withers of the six groups at 8 weeks, the average daily gain being 0.06 inches for each group. The average daily gain in chest circumference at 9 weeks was 0.11, 0.11, 0.09, 0.11, 0.09 and 0.07 inches for groups I through VI, respectively. The differences were not great enough to be significant statistically at 8 weeks.

Measurements of body weight obtained at 24 weeks showed greater variation than did the other measurements. The average daily gains at this period were 1.97, 2.05, 1.91 and 1.96 lb. for groups I through IV, respectively. All four groups were well above the standards reported by Ragsdale (6) for body weight at 24 weeks. Analysis of variance indicated no significant differences among the four groups in body weight during the trial. Very slight differences were observed among groups in measurements of height at withers at 24 weeks. Average daily gains at this period were 0.071 inches for group I and 0.067 inches

TABLE 4

Incidence of Scours During 24-Week Trial (10 calves per group)

	Group I	Group II	Group III	Group IV
No. of cases[a]				
W	0	4	3	1
WB	0	0	0	0
N1	24	22	38	31
N2	1	4	6	6
N3	0	0	1	1
N4	0	0	2	3
Total	25	30	50	42
Av. duration of scours (days)	1.60	0.93	1.30	1.33
No. calves having scours	8	9	9	9
No. calves that received only one sulfathalidine treatment	1	4	2	1
No. calves receiving 2 or more sulfathalidine treatments	1	0	4	2

[a] See table 3.

for groups II, III and IV; the differences were not significant statistically. Growth in height at withers when compared to the standards reported by Ragsdale (6) was well above normal at the end of the 24-week feeding trial. The average daily gains in chest circumference after the calves were on the feeding trial for 24 weeks were 0.12, 0.12, 0.11 and 0.12 inches for groups I through IV, respectively, and there were no statistically significant differences. All groups were above normal when compared to the standards reported by Ragsdale (6) relative to chest circumference after 24 weeks on the feeding trial.

Condition of feces. Data relative to the cases of abnormal feces are summarized at 8 and 24 weeks in tables 3 and 4, respectively. Under the conditions of this experiment there appears to be no relation between the amount of banana meal in the ration of dairy calves and the condition of the feces.

Using the average duration of abnormal feces as a criterion, there appears to be some value in the use of banana meal in the control of this condition. While

it did not decrease the incidence, it did decrease the severity at both 8- and 24-week periods, as indicated by the average duration of cases.

Feed. Indications are that dehydrated banana meal in the grain ration increased the palatability of the rations used in these trials (table 5). While the total grain consumption during the 24 weeks of the trial shows little variation between groups, there was considerable variation among amounts of grain consumed by each group during the first 6 weeks. These figures indicate that during the period when grain feeding was primarily *ad libitum,* the palatability of the grain mix was in direct proportion to the amount of banana meal in the grain mixture.

The total hay consumption during the first 6 weeks showed a tendency for the amount of hay consumed to decrease as the amount of grain mixtures consumed increased during this period.

Little variation was found among groups in the amount of grain consumed per lb. of gain. Group I required 25.0 lb. of grain per lb. as compared to 21.3,

TABLE 5

Grain and Hay Consumption

	Group I	Group II	Group III	Group IV	Group V	Group VI
			(lb.)			
Grain first 6 weeks	375	390	423	586	422	384
Total grain	7105	7349	7124	7209	712	835
Hay first 6 weeks	257	268	237	189	144	207
Total hay	8279	7754	6855	7622	470	388

22.2 and 21.9 for groups II, III and IV, respectively. Hay consumption per lb. of gain was 25.0, 22.5, 21.3 and 23.1 lb. for groups I through IV, respectively.

CONCLUSIONS AND SUMMARY

1. A study has been conducted to determine the value of dehydrated banana meal in the rations of Holstein bull calves. Grain rations containing 5, 20 and 40 per cent of banana meal were fed for a period of 24 weeks. The calves in two additional groups each received 2 and 4 ounces of dehydrated banana meal twice per day mixed with the regular milk feeding during the 7-week period of milk feeding.

2. The inclusion of dehydrated banana meal in grain rations at the levels used or in the milk fed did not increase significantly the rate of growth in terms of body weight, chest circumference or height at withers during the trial. Similarly, the feeding of 2 and 4 ounces of dehydrated banana meal with each of the twice-per-day milk feedings did not affect significantly the rate of growth during the 7-week milk-feeding period.

3. Addition of 5, 20 and 40 per cent dehydrated banana meal to the grain ration or the mixture of 2 and 4 ounces of banana meal with each milk feeding did not reduce significantly the incidence of scours under the conditions of these trials.

4. The dehydrated banana meal did improve the palatability of the rations used. Under the conditions of these trials, banana meal could be used to replace part of the ground yellow corn and rolled oats in the rations of dairy calves.

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THE EFFECT OF PREPARTUM VITAMIN A SUPPLEMENTATION ON THE HEALTH AND PERFORMANCE OF THE YOUNG CALF

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The relationship of the quantitative and qualitative factors of the maternal diet other than extreme deficiencies to the health of the newborn calf has received only limited attention, yet it is apparent that not only is such information of physiological significance but also of economic importance to the dairyman.

Spielman *et al.* (21, 22) and Wise *et al.* (25) were able to demonstrate an increased amount of vitamin A in the blood and livers of newborn calves and in colostrum when the dams were fed supplementary vitamin A during the later stages of the gestation period.

The purpose of the present study was to determine the effect of prepartum vitamin A supplementation on the health and performance of the young calf.

EXPERIMENTAL PROCEDURE

A total of 59 cows of the Brown Swiss, Guernsey, Holstein and Jersey breeds calving in the Cornell University herd from October, 1946, to July, 1947, were allotted to one of four dietary groups 30 days prior to the calculated parturition date. Breed, age, number of previous gestations, length of dry period and previous feeding history were considered in placing cows in a particular dietary grouping. The four dietary groupings were as follows: (a) a basal ration of good quality timothy and clover mixed hay, corn silage and a grain mixture containing 12 per cent crude protein; (b) basal ration plus 1 million I.U. of vitamin A daily in the form of alfalfa leaf meal; (c) basal ration plus 1 million I.U. of vitamin A daily as vitamin A alcohol; (d) basal ration plus 1 million I.U. of vitamin A daily as vitamin A ester. The hay and silage contained an average of 11.8 and 5.0 γ of carotene per g., respectively, as determined by the method of Moore and Ely (10) as modified by Nelson *et al.* (11). Cows calving during October and November received a limited amount of fall pasture. These cows were divided equally among dietary groupings. The dehydrated alfalfa leaf meal contained an average of 114 mg. of carotene[2] per lb. It was hand mixed with the grain and fed once daily. The alcohol and ester forms of vitamin A[2,3] were administered orally once daily in capsules. The potencies of the alcohol and ester forms of vitamin A, as measured by the Evelyn photoelectric colorimeter, were on the average 13.75 and 13.65 mg. of vitamin A per g. of oil.

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[1] Now at the University of Connecticut, Storrs.

[2] It was assumed that 0.25 γ of vitamin A was equivalent to 1 I.U. of vitamin A and that 0.6 γ of carotene was equivalent to 1 I.U. of vitamin A.

[3] The alcohol form of vitamin A was diluted in corn oil and was manufactured to contain 55,000 I.U. of vitamin A per g. The ester form of vitamin A was made up of the ester form of fish liver oil and manufactured to contain 55,000 I.U. of vitamin A per g. Both vitamin A supplements were supplied by Dr. André E. Briod of the Nopco Chemical Company, Harrison, New Jersey.

Calves were left with their dams for the first 2 days after birth. They then were moved to individual pens and raised according to the Cornell dry-calf starter method (23). Milk was fed twice daily from nipple pails. Each calf received its mother's milk up to 8 days of age and thereafter herd milk. Starter and mixed timothy and clover hay were fed free choice, and water was before the calves at all times. Feed intakes and refusals were recorded. Calves were weighed at birth and at weekly intervals thereafter up to 4 weeks of age. Blood samples were drawn from the jugular vein at birth and at weekly intervals thereafter up to 4 weeks of age. Plasma carotene and vitamin A were determined by the method of Kimble (8).

TABLE 1

The effect of the prepartum diet on the carotene content of the plasma of young calves[a]

Breed	No. of calves	Carotene content of plasma (γ/100 ml.) at ages of:				
		Birth	1 week	2 weeks	3 weeks	4 weeks
				Controls		
Holstein	14	2.5 ± 0.4	11.9 ± 1.9	10.5 ± 1.6	12.5 ± 2.0	17.9 ± 3.5
Brown Swiss	2	1.5 ± 1.5	19.5 ± 1.5	14.5 ± 0.5	11.5 ± 5.5	25.5 ± 1.5
Guernsey	2	2.0 ± 2.0	29.3 ± 8.3	25.8 ± 6.3	35.5 ± 14.5	28.5 ± 10.5
Jersey	1	1.0	30.0	38.0	30.0	39.0
All Breeds	19	2.3 ± 0.4	15.7 ± 2.2	14.2 ± 2.2	15.8 ± 2.6	21.1 ± 3.0
				Alfalfa leaf meal		
Holstein	9	2.8 ± 0.8	15.1 ± 3.3	14.4 ± 3.1	15.1 ± 2.4	13.8 ± 1.9
Brown Swiss	2	3.0 ± 0.0	16.0 ± 5.0	14.5 ± 5.5	11.5 ± 2.5	23.5 ± 0.5
Guernsey	2	3.5 ± 1.5	56.0 ± 3.0	27.0 ± 3.0	35.5 ± 2.5	39.0 ± 0.0
Jersey	1	5.0	20.0	14.0	21.0	30.0
All Breeds	14	3.1 ± 0.5	21.9 ± 4.6	16.2 ± 2.4	17.9 ± 2.6	20.0 ± 2.8
				Alcohol form of vitamin A		
Holstein	5	2.6 ± 0.8	16.2 ± 3.6	17.0 ± 2.7	21.8 ± 4.0	21.0 ± 2.6
Brown Swiss	1	2.0	30.0	24.0	54.0	35.0
Guernsey	2	5.0 ± 3.0	39.0 ± 21.0	35.0 ± 21.0	44.0 ± 27.0	32.0 ± 24.0
Jersey	1	3.0	23.0	14.0	26.0	20.0
All Breeds	9	3.1 ± 0.8	23.6 ± 5.2	21.4 ± 4.7	30.8 ± 6.6	24.9 ± 4.7
				Ester form of vitamin A		
Holstein	11	2.7 ± 0.6	13.4 ± 1.5	14.8 ± 2.7	17.1 ± 3.2	17.5 ± 2.7
Brown Swiss	2	1.0 ± 1.0	10.0 ± 2.0	10.5 ± 1.5	17.0 ± 6.0	21.5 ± 8.5
Guernsey	1	6.0	27.0	27.0	35.0	32.0
Jersey	2	3.0 ± 1.0	41.5 ± 3.5	47.0 ± 3.0	52.0 ± 1.0	37.0 ± 7.0
All Breeds	16	2.8 ± 5.0	17.3 ± 2.8	19.1 ± 3.4	22.6 ± 3.8	21.3 ± 2.7

[a] Values given are mean ± standard error of the mean.

The data were treated statistically by analysis of variance as described by Love (9). Only Holstein calves were included in the reported analyses of the data because of the unequal distribution between grouping of calves of the other breeds. However, analysis including all breeds gave essentially the same differences as when only Holstein calves were included. Only those differences significant at the 1 per cent level are included as real differences. In the case of scours, the total number of days each calf was free from scours was divided by the total number of experimental days and the resulting quotient multiplied by 100 to give a percentage. The percentage then was converted to the appropri-

ate angle from Bliss's tables which are contained in Snedecor (17). The angles thus obtained then were used in analysis of variance to test for significance.

RESULTS

The levels of carotene in the blood plasma of the calves are listed in table 1. Holstein calves from dams fed the alcohol form of vitamin A were significantly higher in plasma carotene on the basis of all of the experimental data than calves from dams in the other three dietary groupings. Also, those calves from dams fed the ester form of vitamin A were higher in plasma carotene than those calves from dams fed alfalfa leaf meal. At any one age no significant differences were

TABLE 2

The effect of the prepartum diet on the vitamin A content of the plasma of young calves

Breed	No. of calves	Vitamin A content of plasma (γ/100 ml.) at ages of:				
		Birth	1 week	2 weeks	3 weeks	4 weeks
				Controls		
Holstein	14	3.6 ± 0.5	12.5 ± 1.9	12.8 ± 1.5	10.0 ± 0.9	9.9 ± 6.6
Brown Swiss	2	3.0 ± 0.9	15.7 ± 0.2	11.8 ± 0.8	11.8 ± 3.2	13.3 ± 1.7
Guernsey	2	2.7 ± 0.4	19.6 ± 4.7	11.8 ± 5.0	10.4 ± 1.1	9.0 ± 2.7
Jersey	1	5.3	11.9	11.6	6.9	7.8
All Breeds	19	3.5 ± 0.4	13.6 ± 1.5	12.5 ± 1.1	10.0 ± 0.7	10.1 ± 0.6
				Alfalfa leaf meal		
Holstein	9	4.5 ± 0.7	11.1 ± 1.8	10.3 ± 1.2	10.2 ± 1.0	9.0 ± 0.8
Brown Swiss	1	1.9 ± 0.9	11.2 ± 0.8	7.3 ± 0.4	10.3 ± 0.4	10.9 ± 1.4
Guernsey	2	0.3 ± 0.3	8.1 ± 3.7	9.3 ± 4.5	8.5 ± 0.2	9.0 ± 4.6
Jersey	1	2.0	12.2	8.1	9.0	4.7
All Breeds	9	3.3 ± 0.7	10.7 ± 1.2	9.6 ± 1.0	9.9 ± 0.6	8.9 ± 0.8
				Alcohol form of vitamin A		
Holstein	5	7.2 ± 0.8	16.3 ± 2.7	12.6 ± 2.0	12.8 ± 1.9	13.7 ± 3.1
Brown Swiss	2	8.5	17.6	15.1	16.5	11.3
Guernsey	2	7.9 ± 0.5	13.1 ± 5.3	10.8 ± 2.2	14.1 ± 4.8	11.1 ± 1.2
Jersey	1	5.6	26.3	6.6	11.0	11.9
All Breeds	14	7.3 ± 0.5	16.8 ± 2.1	11.8 ± 1.4	13.3 ± 1.4	12.6 ± 1.2
				Ester form of vitamin A		
Holstein	11	9.9 ± 0.6	17.1 ± 1.9	14.2 ± 1.1	13.7 ± 1.0	13.1 ± 0.7
Brown Swiss	2	5.9 ± 1.1	12.1 ± 1.1	13.8 ± 3.1	10.8 ± 0.8	10.0 ± 0.1
Guernsey	1	3.3	11.6	11.6	9.1	8.4
Jersey	2	8.4 ± 5.7	22.8 ± 5.7	13.7 ± 0.6	13.2 ± 2.7	19.6 ± 3.8
All Breeds	16	8.8 ± 1.6	16.8 ± 1.6	13.9 ± 0.8	12.9 ± 0.8	13.2 ± 0.7

found between groupings. Calves within a grouping varied significantly among themselves during the experimental period and with age (table 1).

Vitamin A levels in the plasma of calves are given in table 2. Calves from dams fed either form of vitamin A were significantly higher in the level of plasma vitamin A based on all the experimental data than calves from control dams or from dams fed alfalfa leaf meal. There were no real differences between either group of calves from dams receiving supplemental vitamin A or between calves from control dams and dams fed alfalfa leaf meal. Significant differences existed at birth and at 3 and 4 weeks of age but not at other specific ages. Calves within an experimental grouping showed significant changes in the blood plasma vitamin A levels with age and among themselves (table 2).

The liveweight gains, as shown in table 3, were significantly higher for those calves from dams fed either form of vitamin A than for those calves from dams fed alfalfa leaf meal or dams fed the basal ration alone. In addition, calves from dams fed alfalfa leaf meal were significantly heavier in liveweight than calves from control dams. At any one age, the only statistical difference was at 4 weeks of age when calves from dams fed the ester form of vitamin A were significantly heavier in liveweight than calves from control dams (table 3).

TABLE 3

The effect of the perpartum diet on the liveweight of young calves

Breed	No. of calves	Age				
		Birth	1 week	2 weeks	3 weeks	4 weeks
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
				Controls		
Holstein	14	95 ± 4	95 ± 4	98 ± 4	101 ± 4	108 ± 4
Brown Swiss	2	98 ± 2	98 ± 3	100 ± 1	106 ± 1	113 ± 0
Guernsey	2	68 ± 22	67 ± 23	66 ± 22	73 ± 28	78 ± 30
Jersey	1	56	56	59	63	72
All Breeds	19	90 ± 4	90 ± 4	92 ± 4	96 ± 4	103 ± 4
				Alfalfa leaf meal		
Holstein	9	98 ± 3	99 ± 3	102 ± 3	108 ± 4	117 ± 4
Brown Swiss	2	80 ± 10	88 ± 16	83 ± 20	92 ± 23	100 ± 20
Guernsey	2	83 ± 6	85 ± 8	87 ± 9	86 ± 15	98 ± 9
Jersey	1	48	49	54	56	57
All Breeds	14	90 ± 4	92 ± 4	94 ± 5	99 ± 4	107 ± 6
				Alcohol form of vitamin A		
Holstein	5	102 ± 6	107 ± 6	108 ± 6	115 ± 8	126 ± 10
Brown Swiss	1	77	79	85	103	104
Guernsey	2	74 ± 2	76 ± 3	80 ± 5	86 ± 3	91 ± 7
Jersey	1	55	55	47	56	65
All Breeds	9	88 ± 7	91 ± 7	92 ± 8	101 ± 8	109 ± 9
				Ester form of vitamin A		
Holstein	11	98 ± 2	101 ± 3	107 ± 3	115 ± 3	124 ± 4
Brown Swiss	2	86 ± 1	87 ± 1	88 ± 1	93 ± 1	103 ± 1
Guernsey	1	80	81	89	95	99
Jersey	2	54 ± 10	59 ± 11	62 ± 12	69 ± 12	76 ± 14
All Breeds	16	90 ± 4	93 ± 4	98 ± 5	105 ± 5	114 ± 5

There were no significant differences found in the feed consumed by the various grouping of calves (table 4). The incidence of scours (days with scours) was significantly lower in those calves from dams fed either form of vitamin A than that observed in calves from basal dams. Calves from dams fed alfalfa leaf meal had significantly fewer days of scours than calves from basal dams (table 4).

DISCUSSION

These data furnish additional evidence of the importance of the prepartum ration on the subsequent performance of the newborn calf. Not only does the prepartum ration influence the storage of vitamin A in the newborn calf and in colostrum (21, 22, 25), but the combination of these two factors apparently

results in superior performance of the young calf as evidenced by the data presented in this paper. Similar data showing statistically higher blood plasma levels and liver storage levels have been found in 30-day-old pigs and lambs from dams fed supplementary vitamin A during the prepartum period (2).

The marked absence of response when carotene in the form of alfalfa meal is added to the maternal ration, in contrast to that found when either the ester or alcohol form of vitamin A is added, is noteworthy. Although earlier data (21, 22) have shown that the feeding of carotene *per se* does influence the carotene content of colostrum and the fetal storage of carotene, no appreciable increase was noted in the vitamin A content as such. Later work by Fountain *et al.* (4) showed that calves from cows on a ration limited to pasture alone had blood plasma vitamin A values equivalent to those obtained in calves from dams fed grain, silage, hay and a vitamin A supplement. Recent studies (1, 7, 16, 19, 20, 24) have suggested that vitamin A is utilized more readily by dairy calves than is carotene. Also, the absorption and utilization of carotene is influenced by the source of carotene and the method of feeding. Crude soybean lecithin recently has been demonstrated (3) to influence the absorption and utilization of carotene and vitamin A in the young dairy calf. Clearly, this whole field needs further study.

TABLE 4

Effect of the prepartum diet on the consumption of milk, starter and hay and the incidence of scours in young calves

Breed	No. of calves	Milk	Starter	Hay	Scours
		(lb.)	*(lb.)*	*(lb.)*	*(days)*
			Controls		
Holstein	14	224.5 ± 5.8	12.5 ± 1.8	4.3 ± 0.9	4.4 ± 1.2
Brown Swiss	2	222.5 ± 6.5	16.0 ± 0.1	8.8 ± 5.7	3.5 ± 0.5
Guernsey	2	154.0 ± 25.0	10.4 ± 0.2	4.8 ± 4.7	7.5 ± 0.5
Jersey	1	165	15.0	5.0	2
All Breeds	19	213.7 ± 7.4	13.3 ± 1.3	4.9 ± 0.9	4.5 ± 0.9
			Alfalfa leaf meal		
Holstein	9	238.2 ± 3.0	17.8 ± 2.9	4.8 ± 1.1	1.6 ± 0.7
Brown Swiss	2	218.5 ± 24.5	14.8 ± 0.3	7.3 ± 3.2	5.0 ± 5.0
Guernsey	2	158.5 ± 3.4	14.9 ± 3.1	2.7 ± 0.4	3.5 ± 3.5
Jersey	1	153.0	18.0	5.5	13.0
All Breeds	14	218.0 ± 9.6	17.0 ± 1.9	4.9 ± 0.8	3.1 ± 0.7
			Alcohol form of vitamin A		
Holstein	5	223.4 ± 9.0	14.0 ± 2.4	8.0 ± 3.3	1.2 ± 1.0
Brown Swiss	1	244.0	9.5	7.5	0.0
Guernsey	2	149.5 ± 9.5	12.4 ± 4.8	5.5 ± 0.5	3.0 ± 3.0
Jersey	1	139.0	12.2	4.5	4.0
All Breeds	9	199.9 ± 14.6	12.9 ± 1.6	7.4 ± 1.5	2.2 ± 1.1
			Ester form of vitamin A		
Holstein	11	242.5 ± 2.8	14.2 ± 2.3	4.8 ± 0.9	0.4 ± 0.4
Brown Swiss	2	236.5 ± 6.0	14.8 ± 4.2	9.9 ± 6.1	0.0 ± 0.0
Guernsey	1	161.0	16.1	11.0	0.0
Jersey	2	168.0 ± 0	17.2 ± 9.3	5.2 ± 0.7	0.0 ± 0.0
All Breeds	16	227.3 ± 7.9	15.4 ± 1.9	5.9 ± 1.0	0.3 ± 0.1

The same response of calves to either the ester or alcohol forms of vitamin A fed to their dams is in agreement with other published information. Ross *et al.* (16) found no appreciable difference in blood plasma levels of vitamin A in Holstein heifers when the ester or alcohol form of vitamin A was fed in equivalent amounts. Regardless of the ration or the form of the supplementary vitamin A fed during the prepartum period, practically all of the vitamin A contained in the colostrum or milk was present in the ester form (14, 22). The prepartum feeding of supplementary vitamin A resulted in increases in the ester form of vitamin A occurring in the blood plasma (15). The increase in the ester form of vitamin A occurred when either the ester form or alcohol form of vitamin A was fed. In newborn and young calves the blood plasma vitamin A was predominately in the alcohol form, while the vitamin A contained in the liver was largely in the ester form. Limited data indicated that these relationships existed regardless of the form of supplementary vitamin A fed the dam.

The apparent absence of beneficial results from postnatal supplementary vitamin feeding (5, 6, 12, 13, 18) under so-called normal feeding conditions and the beneficial results reported herein in prepartum vitamin A supplementation would indicate the need for additional work with emphasis on prepartum nutrition. However, the limited number of calves, especially as to breeds other than Holsteins, would indicate the need for additional work before application of these results can be realized.

SUMMARY

The effects of feeding 1 million I.U. of vitamin A, in the form of dehydrated alfalfa leaf meal, the ester form of vitamin A and the alcohol form of vitamin A, daily to cows 30 days prepartum on the blood plasma levels of carotene and vitamin A, liveweight changes, feed consumption and incidence of scours of calves from birth to 28 days of age have been studied.

Carotene levels in the blood plasma of calves from dams fed the alcohol form of vitamin A were significantly higher during the experimental period than calves from dams in the other dietary groupings. Also, calves from dams receiving the ester form of vitamin A were significantly higher in plasma carotene than calves from dams fed alfalfa leaf meal.

Plasma of calves from cows fed vitamin A as either ester or alcohol was significantly higher in vitamin A than that of calves from the basal dams or those fed alfalfa leaf meal.

Greater liveweight increases were observed in calves from dams fed either form of vitamin A than in calves from dams fed the basal ration alone or with alfalfa leaf meal. In addition, calves from dams fed alfalfa leaf meal made greater increases in liveweight during the entire experimental period than calves from basal dams.

There were no statistical differences found in feed consumption.

Incidence of scours was significantly lower in calves from dams fed either form of vitamin A than that observed in calves from control dams. Calves from dams fed alfalfa leaf meal had fewer cases of scours than calves from control dams.

ACKNOWLEDGMENTS

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THE RELATIONSHIP BETWEEN TYPE RATINGS OF AYRSHIRE FEMALES AS YOUNG HEIFERS AND AS COWS[1]

GEORGE HYATT, JR.,[2] W. J. TYLER,[3] AND C. T. CONKLIN[4]

The type and over-all eye appeal of an animal always has been an important factor in selecting breeding stock. Livestock breeders like to compare the type of their cattle with that of their neighbors at home, at fairs, and at shows and exhibits. Many breeders and educators argue that the type conformation of a dairy animal bears little relationship to her usefulness as a producer. They argue that a level rump, a cleancut appearance and a well balanced and attached udder are of little importance in selecting and breeding cattle. Some have said that the show ring has done more harm than good in the development of our breeds. Others breeders and educators argue just as strongly that type and production go hand in hand, and stress proper type conformation, even to the point where some production may be sacrificed. Despite the arguments each way, the fact remains that dairymen and livestock breeders of all kinds usually try to seek the best type cows for additions to their herds and are willing to pay higher prices for these "typy" animals. Type classification programs have been adopted by the five major dairy cattle breed associations and, although these programs still need to be strengthened, they represent the most scientific approach yet devised for the estimation and use of type in a breeding program.

A study of the inheritance of official type ratings of Ayrshire cows was made by Tyler and Hyatt (3), who found heritability to be approximately 30 per cent. These same workers found the repeatability between type ratings given to the same cow by different inspectors to be 0.55. When the ratings on a cow were made by the same inspector the repeatability figures were 0.73, 0.82 and 0.62 for three inspectors.

Johnson and Lush (2) studied yearly type ratings of Holstein-Friesian females from 6 months of age to maturity. They state that "ratings made under one year of age were somewhat less repeatable than those made at older ages" and that "the increase in permanancy of ratings after one year of age was small." When they omitted ratings on heifers less than one year of age, they reported a correlation of 0.34 between consecutive ratings when the ratings were made by nationally-known dairy cattle judges.

EXPERIMENTAL PROCEDURE

If type is heritable and important to breeders of dairy cattle, it is desirable that dairymen should be able to cull the poorest type animals from their herds

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at as early a date as possible to save feed, labor and money. This raised the question as to whether the type classification program, as applied to cows, also could be used for evaluating the type of young heifers. Therefore, a cooperative project was inaugurated in 1942 by the West Virginia Agricultural Experiment Station and the Aryshire Breeders' Association to determine the repeatability of heifer classifications at 6-month intervals and the correlation between the several classifications of a heifer and her classification after freshening. The Ayrshire cattle at the West Virginia Agricultural Experiment Station are known as the Reymann Memorial Herd and have been on a long-time, carefully controlled breeding project for more than 25 years. This project does not permit the culling of any normal females until they have completed at least one lactation.

During the period from October, 1942, through April, 1948, 102 Ayrshire heifers were classified every 6 months, starting at about 6 or 12 months of age and continuing in some instances to 5 years of age. The type ratings were made in each instance by an official inspector designated by the Ayrshire Breeders' Asso-

TABLE 1

The average number, mean and standard deviation of ratings on 102 Ayrshire females classified as heifers and as cows

	Ratings made as:		
	Heifers	Cows	Combined
Number of animals	102	102	102
Average number of ratings	4.0	2.4	6.4
Mean	3.15	3.09	3.13
Intra-animal standard deviation	0.62	0.65	0.66

ciation. The inspectors classified each heifer into one of five grades (Excellent, Very Good, Good Plus, Good and Fair) similar to the classification program of adult animals. In no instance did they have knowledge of the previous classification of the animals until the entire job had been completed. In order to facilitate the analysis of the data the official grades were coded, using 5 for Excellent, 4 for Very Good, 3 for Good Plus, 2 for Good, and 1 for Fair.

RESULTS

Variations in individual ratings of heifers and cows. The 102 females in the study had a total of 407 ratings before calving and 243 ratings following calving. These two groups of ratings were analyzed by the analysis of variance and the intra-animal standard deviations were computed. The results are shown in table 1. The variation in the ratings of animals before first calving was approximately the same as the variation in ratings given to the same animals after they had calved. These standard deviations also are similar to the intra-cow variation (standard deviation = 0.64) reported by Hyatt and Tyler (1) for 101 Ayrshire cows classified two times or more.

In terms of range (difference between highest and lowest ratings on an animal) 77 of the heifers, or 75 per cent, varied one grade or none from the time they

first were classified until first freshening, while 57 animals, or 60 per cent, varied one grade or none in their ratings from 6 months to 4 or 5 years of age. Before first calving 24 per cent of the animals had a range of two grades in their ratings, while 38 per cent of the animals showed a range of two grades when all ratings were considered. Only one heifer varied three grades before first calving, while six animals varied three grades for all ratings.

A review of the classification ratings of the six animals that ranged three grades showed that one heifer (no. 789) was classified Very Good at 12 months of age, Good Plus at 18 and 24 months, Fair at 30 months, and Good following freshening. Each time she was classified her feet and legs were Fair or Good and her final rating depended upon how severe a cut the inspector thought should be placed on that defect. Heifer no. 737 classified Good Plus at 6 and 18 months, Very Good at 12, 24 and 30 months. The first two times following freshening she classified Very Good, then dropped to Good and 6 months later classified Excellent. At the time she was classified Good she was dry and showed very little udder development. However, during heavy lactation following freshening, she showed a large and well-balanced udder and was classified Excellent. Heifer

TABLE 2

Average classification score of 33 heifers at 6, 12, 18 and 24 months and 73 heifers at 12, 18 and 24 months of age

No. of heifers	Average classification rating			
	6 mo.	12 mo.	18 mo.	24 mo.
33	2.91	3.12	3.10	3.06
73		3.15	3.18	3.21

no. 729 classified Very Good at 12 months, Good Plus at 18 months and Good at 24 months. Following freshening, she was classified twice as Fair before leaving the herd. In each instance she was criticized for lacking in fore udder development and attachment. This fault appeared to become progressively worse as she became older. Heifer no. 727 classified Good Plus at 6 and 30 months, Very Good at 12 months and Good at 24 months. The first time after freshening she classified Good Plus but was dropped to Fair at the next classification. In each instance she was criticized for being sickle-hocked, and this condition became more defective as she became older. The other two animals that varied three grades were criticized for similar faults.

Effect of age on average type ratings of heifers. There were 33 heifers that were classified at 6, 12, 18 and 24 months of age. The averages of the ratings at the four different ages were approximately the same. This also was true of a group of 73 heifers classified at 12, 18 and 24 months of age. The results are shown in table 2.

These results seem to be contrary to the popular belief of many dairymen that most heifers appear to look typier at about 6 months of age than at any other time before freshening.

A comparison of the classification ratings of heifers before first calving with

TABLE 3

The correlation coefficients (r) between the various classification ratings of heifers at 6, 12, 18, 24 and 30 months of age and between these ratings and the 1st, 2nd and average of 1st and 2nd after-calving (AC) ratings

Age	Age											
	6 mo.		12 mo.		18 mo.		24 mo.		30 mo.		All before calving	
	No.	r	No.	r	No.	r	No.	r	No.	r	No.	r
12 months	42	.30										
18 months	39	.10	89	.47[2]								
24 months	42	.33[1]	92	.31[2]	94	.38[2]						
30 months	20	.40	66	.48[2]	67	.57[2]	73	.54[2]				
1st AC	42	.33[1]	92	.31[2]	93	.22[1]	102	.25[1]	73	.35[2]	102	.37[2]
2nd AC	29	.23	67	.27[1]	69	.29[1]	76	.18	60	.45[2]	76	.40[2]
Av. 1st and 2nd AC	29	.35[1]	67	.36[2]	69	.40[2]	76	.29[2]	60	.40[2]	76	.44[2]

[1] $P < .05 > .01$
[2] $P < .01$

their ratings after freshening. Correlation coefficients were computed between the various ratings before calving and also between the ratings before first calving and the ratings after calving. These coefficients are shown in table 3. Because of the limited number of animals in this experiment, the sampling errors are large. Thus, no particular age group had a significantly higher value for predicting after-calving ratings. The correlation between the average of the several ratings of each heifer before first calving and her first and second ratings after calving were 0.37 and 0.40, respectively. This is slightly smaller than the repeatability figure of 0.55 between the ratings of cows classified by different inspectors (1).

The regression of average after-calving ratings on the average ratings before calving was 0.45. An example of the use of this regression may be shown as follows: The type ratings of the heifers before first calving were compared with their ratings after calving by placing all the heifers in groups according to their average classification score prior to first calving. This average group score before freshening was then compared with the average classification score of the same group after first calving. The average score was computed by giving each animal 92.5 points for each Excellent rating, 87.5 for Very Good, 82.5 for Good

TABLE 4

Average classification ratings by groups before and after calving

Average rating of animal before calving by groups	No. of animals	Average of each group before calving	Average of each group after calving
Below 80.5	14	78.6	80.1
80.5–82.5	34	82.0	81.5
82.6–85.0	31	84.4	83.0
Above 85.1	23	87.1	84.5
Total	102	83.4	82.4

Regression of average after-calving rating on average before-calving rating = 0.45.

Plus, 77.5 for Good, and 72.5 for Fair, and then adding up the points and dividing by the total times classified. Those animals that averaged below 80.5 were placed in one group, those whose average ratings were between 80.5 and 82.5 fell in the second group, while the other two groups were comprised of animals whose average classification scores were between 82.6 and 85.1 and higher. The average of each group before calving is given in table 4 and can be compared with the group average of the same animals after they have freshened. The heifers that were in the two lower groups, as far as type was concerned, still had the lower classification average after they were rated as milking cows, but the averages were closer to the average of all the animals. Those animals in the upper groups were still above average when they were classified as 3-, 4- and 5-year old cows, but the two group averages were nearer the average of all groups.

DISCUSSION

The results of this study have indicated that the relationship between any classification rating on Ayrshire heifers before calving and a rating after calving is about 0.3. In addition, correlations between combinations of ratings before first calving and ratings after calving are approximately 0.4. In a previous study on this herd (1) the repeatability of classification ratings for the same cow was estimated to be 0.55. Thus, while ratings on heifers before first calving are indicative of after-calving ratings, they are not as reliable as after-calving ratings in predicting other after-calving ratings. These results are similar to the study on classifications of Holsteins reported by Johnson and Lush (2).

The variation between ratings on the same animal before calving was similar to the variation that was found between ratings on the same cow. This presents the same problem in classifying heifers as was found in the classification of cows (1), namely, a means of identifying and adjusting for those factors which cause ratings to vary during the life of the animal. Since nine different inspectors classified the animals during the course of this experiment, an undetermined part of the variation in the ratings was caused by differences of opinion of the inspectors. In other instances, the differences in ratings were causes by changes in the condition and/or type of the animals. At the present time the basis used for the classification of uncalved heifers is the same that has been applied in classifying cows. This may be the wrong approach in classifying heifers. Research on the development of the features of type in growing heifers may reveal the essential parts of the heifer that contribute to her type as a mature animal. Perhaps with more experimental work, detailed records and frequent consultations on the part of inspectors with one another, it may be possible to reduce the variation in heifer classification that is due to differences in inspectors and to inaccuracies in weighing the good and poor points of heifers during their early development.

The standard deviation of all ratings on the heifers before their first calving was 0.78. This is smaller than the standard deviation (0.94) for all ratings on cows in the same herd during the same period. Part of the smaller variation

in ratings on heifers may be attributed to the observation that heifers generally do not show the extreme faults in feet, legs and udders that cows do. In addition, many inspectors hesitate to classify heifers Fair or to classify them Excellent because later development of the heifer may make their decision look absurd.

SUMMARY

One hundred and two Ayrshire heifers at the West Virginia Agricultural Experiment Station have been rated for type starting at 6 or 12 months of age and at 6-month intervals thereafter until 4 or 5 years of age. The intra-animal standard deviation was 0.62 when the ratings were made before first calving, and 0.65 when they were given after calving.

In terms of range, five animals (4.9 per cent) were classified the same whether rated as heifers or milking cows, 52 (51.0 per cent) varied one grade, while 39 (38.2 per cent) varied two grades and six (5.9 per cent) varied three grades.

There were 33 heifers classified at 6, 12, 18 and 24 months of age and 73 heifers classified at 12, 18 and 24 months. The average of the ratings at the different ages was approximately the same.

The correlations between the average of the several ratings of each heifer before first calving and the first and second ratings after calving were 0.37 and 0.40, respectively.

The average type ratings of the heifers before calving were compared with the average ratings after calving by placing the heifers in four groups (below 80.5, 80.5 to 82.5, 82.6 to 85.0, above 85.1) according to the average of their ratings before first calving. The low group remained the lowest following calving, while the high group still maintained the highest average score, but both averages were closer to the general mean. The two middle group averages changed very little following freshening.

With improvement in and standardization of classification methods, particularly for heifers, the classification program may become valuable in helping breeders to cull the poorest type individuals from their herds at an early age.

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ASSOCIATION ANNOUNCEMENT

FORTY-FOURTH ANNUAL MEETING
UNIVERSITY OF MINNESOTA
JUNE 21–23, 1949

REGISTRATION AND HOUSING

Registration headquarters will be in Coffman Memorial Union, Minneapolis Campus. Rooms to accommodate about 250 persons will be available in dormitories on the St. Paul Campus. Families with children will have prior claim to these rooms. Approximately 400 rooms have been reserved in Twin City hotels. A return card relative to advance registration and housing will be sent to members by the Association Secretary in May.

LOCATION OF GENERAL AND SECTION MEETINGS

All meetings will be held in or in close proximity to Coffman Memorial Union, Minneapolis Campus, University of Minnesota.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings and committee meetings before, during or after the regular sessions will please contact Elmer L. Thomas of the Division of Dairy Husbandry, University Farm, St. Paul. Reservations for special breakfasts and luncheons will be made upon request. It will be appreciated if all such requests are made known by June 1.

PROJECTION EQUIPMENT

While it is desired that the use of slides be minimized, projectors for both standard and $2'' \times 2''$ slides will be available in all lecture rooms. Requests for use of such equipment should be made to section chairmen as soon as possible.

ADDITIONAL INFORMATION

Additional material containing information on registration, housing, entertainment, recreation, etc., will be sent to each member about April 15, 1949.

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ABSTRACTS OF LITERATURE

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BOOK REVIEWS

190. Principles of food freezing. W. A. GORTNER, F. S. ERDMAN AND NANCY K. MASTERMAN. John Wiley & Sons, Inc., New York, N. Y. 281 pp. $3.75. 1948.

Although this book contains comparatively little directly about dairy products, the chapters concerned with the engineering of food freezing contain considerable material on the design and operation of refrigeration facilities which should be of interest to people in the dairy industry because of the straightforward presentation. For those who are operating locker plants in conjunction with dairy enterprises or are marketing frozen foods, considerable information on processing, holding and utilization of miscellaneous frozen foods is presented. The book is not an exhaustive treatise but rather a useful summary of the frozen food industry written in reasonably non-technical language. The format is good and the indexing appears to be better than one sometimes finds in a book of this type. F. E. Nelson

191. Food plant sanitation. M. E. PARKER. McGraw-Hill Book Co., Inc., New York, N.Y. 447 pp. $6.00. 1948

Although written for the entire food industry, this book contains considerable information directly applicable to the dairy industry. All phases of sanitation are covered, from the design of the plant and the sanitary quality of the water supply to the placing of the food in a package which will provide good sanitation during marketing. Detergency and insect and rodent control are treated at some length. The establishment and maintenance of sanitary practices and the proper training of personnel are discussed. An appendix section on "Excerpts from U. S. Laws and Regulations" is included. This book should be of considerable value to those concerned with sanitation problems in the dairy industry. F. E. Nelson

192. The chemistry and technology of enzymes. HENRY TAUBER. John Wiley & Sons, Inc., New York, N. Y. 550 pp. $7.50. 1949.

This volume supersedes *Enzyme chemistry* and *Enzyme Technology* by the same author; therefore, the first part is concerned with the chemistry of enzymes and the second part with their technology. This treatment has led to some duplication—almost replication in several instances—while pertinent material has not been included in other cases, probably to keep this book at a reasonable size. A considerable mass of material is presented and citations to the original literature are numerous. Applications to a wide variety of fields are included. The chapter on "Enzymes in Dairy Products" includes a short and relatively inadequate description of the milk phosphatase enzyme and its use for control of pasteurization. No material on milk lipase is presented. Cheese ripening is discussed in 6 pages, probably adequate space for this type of publication if the choice of material had been better. Prevention of mold growth on the surface of Cheddar cheese is ascribed to surface salting. The book contains quite a few errors in the naming of organisms and the same organism is discussed under more than one name in some instances. This book probably is another indication that many fields formerly considered quite specialized are becoming so complex that only rarely will one person be able to encompass with complete adequacy all areas which should be included in a discussion of the entire field. F. E. Nelson

193. Milk and milk processing. B. L. HERRINGTON. McGraw-Hill Book Co., Inc., New York, N. Y. 343 pp. $3.75. 1948.

This book was written as a text for a sophomore course in general dairy manufacturing and thus presupposes a knowledge of general chemistry. As would be expected from the background of the author, the chemical approach is emphasized in presentation of the material. The first part of the book is concerned with the composition of milk and the nature of the different constituents. Chapters on microorganisms, pasteurization, creaming and cream separation, and homogenization are followed by discussions of the various dairy products. Chapters on the Babcock test and lactometry follow. The index seems adequate for a book of this type. The material is pre-

sented logically and in an easily understood form. This book should be very useful not only as a text for a course in general dairy manufacturing but also as a reference book for plant operators with reasonably good technical background.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

194. Field studies on bovine venereal trichomoniasis effects on herds and efficacy of certain practices in control. D. E. BARTLETT AND G. DIKMANS. Bureau of Animal Industry, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, **10,** 34: 30–39. Jan., 1949.

An analysis of the effect of trichomoniasis upon the breeding efficiency of a herd is made with detailed protocol of 2 affected herds. A graphical presentation of reproductive records in each herd clearly shows the progressive nature of the disease in the herd and the rapid recovery following institution of control measures. Effective control measures are discussed in detail and a graphic outline is given to emphasize the important steps in the hygienic breeding program. The basis of the program is accurate diagnosis of the infectious state of each animal in the herd, use of clean sires and sexual rest for infected females. Eight of nine herds following this program have recovered satisfactorily from trichomoniasis.

E. W. Swanson

195. Further observations on experimental treatments of Trichomonas foetus infection in bulls. D. E. BARTLETT. Bureau of Animal Industry, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, **9,** 33: 351–359. Oct., 1948.

The intravenous administration of NaI was successful in eliminating *T. foetus* infection in 2 of 8 trials. Other treatments with iodides are reviewed and a seasonal trend in their efficiency is suggested. Intrapreputial insufflation with a silver protein powder and direct application of a trypoflavin ointment were ineffective. Topical application of a German-manufactured product containing trypoflavin, Surfen A, triethyleneglycol and a condensation product of ethylene oxide and water was effective in 7 of 8 trials. The latter was the only effective topically applied material encountered.

E. W. Swanson

196. A nonantigenic allergic agent for intradermal brucellosis tests. H. E. OTTOSEN AND N. PLUM. State Serum Laboratory, Copenhagen, Denmark. Am. J. Vet. Research, **10,** 34: 5–11. Jan., 1949.

A method for the preparation of an extract from *Brucella abortus* by acid hydrolysis is described in detail. The extract did not promote complement-fixing antibodies following intravenous injection, but it elicited strong allergic reactions in *Brucella*-infected animals. Good correlation was observed between the intradermal test in cattle and the blood agglutination test.

E. W. Swanson

197. Studies on the pathogenicity of Brucella suis for cattle I. F. V. WASHKO, L. M. HUTCHINGS AND C. R. DONHAM. Purdue Univ., Lafayette, Ind. Am. J. Vet. Research, **9,** 33: 342–350. Oct., 1948.

Of 9 cows fed aborted swine fetuses or exposed to *B. suis*-infected swine, *B. suis* was recovered from only one at parturition. Cows previously exposed to *B. suis* did not show significantly greater resistance to *Brucella abortus* than did unexposed control animals. Attempts to establish *B. suis* infection in the bovine udder were successful following introduction of the organisms into the teat canal by injection or by applying to the teat orifice, but were unsuccessful following application of *B. suis* to abraded udder skin, feeding of infected material or exposing of cows to infected swine in a small pen. Acute mastitis resulted from the introduction of *B. suis* into the mamary gland. Elimination of the infectious organisms in the milk was persistent and intermittent throughout the lactation.

E. W. Swanson

198. The Brucella abortus ring test. P. A. BRUHN. Royal Veterinary and Agricultural College, Copenhagen, Denmark. Am. J. Vet. Research, **9,** 33: 360–369. Oct., 1948.

A short review of the development of the ring test is presented. A specific *B. abortus* antigen dyed with hematoxylin is mixed with milk. The distribution of color between the cream and skim milk layers constitutes the basis of the test. The interference of the Schern-Gorlisch reaction (used to detect heated milk) with the ring test is discussed. False positive tests are considered a combination of the S-G reaction and nonspecificity of the antigen. Extensive field use of highly specific antigens showed the ring test to be about 80 to 95% accurate. Greater accuracy was secured with pooled herd milk than with individual-cow samples, due largely to variations in creaming of the latter. The development of a highly specific antigen for the ring test is discussed briefly. This antigen gave positive results on pooled milk diluted 1:128, compared to a sensitivity of only 4-fold dilution with a whey agglutination test. Evidence is presented that consistently positive ring tests indicate infection even in the presence of extremely low blood

titers. The use of the ring test is proposed as a means of more effectively applying a brucellosis eradication program on an area basis.

E. W. Swanson

199. Effect of sulfamethazine on certain infections of the bovine mammary gland. O. W. SCHALM, R. A. BANKOWSKI, R. W. ORMSBEE, AND T. W. BROWNE. Univ. of California, Berkeley. Am. J. Vet. Research, **10,** 34: 56–62. Jan., 1949.

Sulfamethazine was given intravenously, followed by 3 daily oral doses to a group of 10 cows infected with *Staphylococcus aureus, Aerobacter aerogenes,* and *Streptococcus agalactiae.* No significant change in the udder infection or character of the milk occurred. Levels of sulfamethazine in the milk were considered too low, so a series of 6 semi-daily intramammary infusions were given to 7 infected cows. Infection persisted in all of the cows following treatment. Milk was more abnormal following treatment than before, although production was not decreased. Oral treatment of 8 dry cows was most successful in establishing desired udder concentrations of sulfamethazine, but it was not effective in eliminating *S. aureus* and *A. aerogenes* infections. Treatment of acute and chronic cases of *A. aerogenes* and *Pseudomonas aeruginosa* mastitis with sulfamethazine also was ineffective.

E. W. Swanson

200. Bovine mastitis. Economic importance of the disease—methods of detection, control and prevention. G. R. SPENCER. Univ. of Wisconsin, Madison. Milk Plant Monthly, **37,** 12: 35–36, 77. Dec., 1948.

Mastitis, the most costly of all diseases related to the dairy cow, is the most puzzling problem that confronts the dairy industry today. Chronic mastitis which may survive for months and years usually is caused by streptococci, whereas acute mastitis which lasts only a few days commonly is caused by staphlycocci and coliform organisms. Although this disease is of no serious threat to public health, the cause of this inflammation must be detected and treated, since health-endangering hemolytic organisms may infect the udder. Proper pasteurizaiton has done much to eliminate this threat.

The microscopic body-cell count and the catalase tests are used widely in the laboratory for detecting mastitis. Herdsmen must rely on physical examination of the milk and animals for detecting the disease. Segregations, sanitation, disinfection and proper milking procedures aid most in controlling mastitis. However, treatment of the animal with penicillin and sulfonamides under the direction of a veterinarian has aided in effecting control in certain types of mastitis.

J. A. Meiser, Jr.

201. The results of a mastitis herd survey in the N. E. midland area during 1946–1948. D. L. HUGHES. Vet. Record, **60,** 50: 659. Feb., 1949.

In the process of research on penicillin therapy for mastitis, the author examined bacteriologically milk samples from 19 problem mastitis herds involving a total of 591 cows. The types of infection found were as follows: *Staph. pyogenes,* 120 cows or 20.3%; *Str. agalactiae,* 91 cows or 15.2% and *Str. dysgalactiae,* 24 cows or 4.0%. In a few herds a variety of organisms was found such as diphtheriod bacilli, unidentified streptococci and micrococci. The author suggests that the unexpected comparatively low level of *Str. agalactiae* infections in the herds surveyed may be due to the rather widespread use of penicillin therapy in that area or possibly variations because of locality. In a similar investigation undertaken in different counties in 1944–45, a *Str. agalactiae* incidence of 59.3% was found.

R. P. Neidermeier

Also see abs. no. 265, 266.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

202. Packaging of print butter. T. A. NICKERSON AND S. T. COULTER, Univ. of Minnesota. Natl. Butter Cheese J., **40,** 1: 26–27, 52, 54–57. Jan., 1949.

This study was made to obtain information on the protection furnished print butter by various combinations of wrapping material against such factors as absorption of odors, oxidation, shrinkage, physical damage and deterioration in the consumer's refrigerator. The heat-sealed pliofilm and cellophane gave the best protection against absorption of odors. However, no one wrapper could protect the butter from all foreign odors. The wrapping material that protected butter from light provided the best security against deterioration caused by oxidation. The degree of oxidation of the butter was determined by flavor scores, as well as by the aldehyde and peroxide values of the fat.

Four 0.25-lb. prints of butter suffered a greater loss due to shrinkage than a 1-lb. print under similar conditions. Foils and pliofilm both gave satisfactory proetction against shrinkage as did parchment + plioform + carton. The greatest resistance against physical damage to the butter was afforded by the cardboard cartons. Wrapping materials such as cellophane, pliofilm or parchment gave little protection against external phy-

sical force. Under home storage conditions foil and parchment + carton gave about equal protection to the butter. H. E. Calbert

203. Let's sell butter! Roy Robichaux. Western-United Dairy Co., Chicago, Ill. Milk Plant Monthly, **38,** 1: 70–72. Jan., 1949.

A five-point program for promoting the sale of butter is outlined as follows: (1) Use cream of sufficient quality to produce at least a 90 score butter. (2) Use proper processing methods and technics so that the best possible butter is produced. (3) Use appealing efficient packaging methods that will insure the product against outside contamination. (4) Police the consumer outlets to insure that the product is fresh and wholesome when it reaches the consumer. (5) Use only butter of 91 score or better when storing for future consumer use. J. A. Meiser, Jr.

204. De invloed van het vetgchalte van den room op de stevigheid, het vochtgehalte en andere eigenschappen van de boter. (The influence of the fat content of the cream upon the consistency, the water content and other properties of the butter.) (English summary.) H. Mulder. Rykslandbouwproefstation, Hoorn, Holland. Landbouwkund. Onderzoekingen, **52,** 6C: 268–276. 1946.

Butters were made from creams with different fat content under the same temperature conditions and other conditions as much the same as possible. Fat contents of 25–50% were compared with 8–12%. The thin cream took a much longer churning time, butter granules grew very slowly and needed a long working time. These butters contained more moisture and buttermilk than the other butters. There was no difference in hardness.

The fat content of the buttermilk was about the same, causing high fat losses in the cases of thin cream. The phospholipid content of the buttermilk was about proportional to the fat content of the cream. More fat could be separated out of the buttermilk from the thin cream. This cannot be explained by the different phospholipid content. After separating the buttermilk, the fat loss with thin cream still remained higher.

A. F. Tamsma

205. Onderzoek naar de mogelykheid van verbetering van de stevigheid van zomerboter. (Investigation into the possibility of increasing the firmness of summer butter.) W. Adriani and A. F. Tamsma. Laboratorium der Coop. Fabriek van Melkproducten, Bedum, Holland. Landbouwkund. Onderzoekingen, **47,** 12G: 941–1024. 1941.

Experiments were made to ascertain how the firmness of butter (measured at 18° C.) is influenced by the manufacturing process. Souring at 18° C. had no advantage against souring at normal temperature (14° C.); sometimes it caused the fat content of the buttermilk and the water content of the butter to be too high. Both too cold and too warm wash-water led to softer butter. Souring, churning and washing at about 14° C. gave the best results.

To get more insight, the crystallizing of butterfat was studied by the dilatometric method. During cooling, a very pronounced undercooling phenomenon takes place. By cooling butterfat gradually and afterwards increasing the temperature little by little, in the diagram representing the quantity of solid phase as dependent upon the temperature, a loop is described so that the quantity of solid phase at a given temperature is higher when this temperature is reached from a lower temperature rather than from a higher temperature. Cooling in steps leads to a smaller quantity of solid phase. At a given temperature, for instance 18° C., one gets the largest quantity of solid phase by cooling melted butterfat immediately to a lower temperature, e.g., 13 to 14° C., and raising the temperature to 18° C. only after the fat has remained a sufficiently long time at the lower temperature.

The theory was set up that firmness of the butter depends in the first place on the quantity of solid phase (crystallized butterfat) and in the second place on the structure of this solid phase. From this theory it follows why souring, churning and washing at temperatures above 14° C. had an adverse effect on the firmness of butter (measured at 18° C.) and why cold wash water led to softer butter. The results of the present investigation could be explained in this way. Also, the results of some experiments which are described in the literature, but which could not be explained in another way, could be interpreted easily by this theory and the fundamental facts mentioned previously.

One must prevent the butter from reaching (even temporarily) a higher temperature. Too high temperatures proved very disadvantageous and cooling to a lower temperature after exposure to high temperatures did not restore the original firmness.

The temperature of the butter is of great importance for the firmness, because the temperature coefficient is large, a difference of 10% in firmness corresponding to a temperature difference of only 0.25° C. A. F. Tamsma

206. Onderzoek naar de mogelykheid van verbetering der consitentie van winterboter. (Investigation into the possibility of improving the texture of winter butter.) (English and German summary.) W. Adriani and A. F. Tamsma.

Laboratorium der Cooperatieve Fabriek van Melkproducten, Bedum, Holland. Landbouwkund. Onderzoekingen, **52,** 1G: 1–24. 1946.

A good texture of winter butter may be obtained in choosing the right manufacturing process, taking care that the structure leading to brittleness is destroyed and that the percentage of crystallized fat in the butter at a given temperature, e.g., 13° C., is as low as possible, as a high percentage of crystallized fat goes together with a high hardness of the butter.

The first point is effected by working at low temperature, which can be reached by washing the butter three times with washing water of 9° C. or a little lower still. The second point is effected by cooling the cream in successive steps. In a laboratory investigation keeping butterfat at different temperatures and determining the percentage of crystallized fat by the dilatometric method, it was established which temperature steps were the most effective for a good texture of the butter at 13° C. The results obtained were confirmed by experiments on a technical scale in the factory, proving the following manufacturing process satisfactory. The pasteurized cream is cooled at 14° C. After some hours the temperature is raised to 22° C. and maintained during an hour. Then the cream is cooled to 14° C. and soured at this temperature overnight. The ripened cream is churned at 14° C. and the butter washed and worked as described above.

A. F. Tamsma

207. Verschillen in stevigheid by Zomerboters door verschillende fabrieken bereid. (Differences in firmness of summer butters, produced by different factories.) (English, French and German summaries.) W. Adriani and A. F. Tamsma. Laboratorium der Cooperatieve Fabriek van Melkproducten, Bedum Holland. Vakgroep Boterindustrie. The Hague, Holland. 23 pp. 1946.

Determination of the firmness of summer butter at 18° C., rather than at 13° C., is advocated for two reasons: (a) The firmness of summer butter is of most interest at the higher temperature. (b) At 13° C. the systematic differences between the factories were partly and sometimes completely covered by the great fluctuations that, on the strength of theoretical considerations derived from study of butterfat, were expected to occur in a much smaller degree at higher temperature. Statistical examination of all data available at different factories showed that if differences in fat composition were taken into account, there were still some factories producing butter which on an average was firmer, other which was softer than that corresponding with the calculated line of regression. These differences could be explained sufficiently by differences in method of manufacture according to the facts established by the investigations of the authors. The composition of the milk fat affected the firmness of the summer butter of different factories much more than did the method of manufacture.

A. F. Tamsma

CHEESE

A. C. DAHLBERG, SECTION EDITOR

208. Smaak en smaakstoffen van kaas. (Taste and taste compounds of cheese). H. Mulder. Landbouwkund. Tijdschr. **59,** 706/708: 181–187. March/May, 1947.

After an introduction on the history of different kinds of cheese, the author comes to a description of taste, particularly taste of cheese. The different components of cheese, as far as they contribute to the taste, are discussed. The equilibrium and cooperation of the taste components are considered important. Nomads probably made the first cheese for army supply. Afterwards educated peoples developed the taste. Later on the existence of different kinds of cheese was governed more by economic than by taste reasons.

The simple differentiation of taste into sweet, sour, salt and bitter is found not adequate, taste being much more complicated. The taste of cheese has a general component but is very variable, because of the many other components. Fat, protein, milk sugar, with their degradation products, milk salts, salt and water all contribute to the taste. Fat is very important as by hydrolysis fatty acids are formed, causing probably a main part of the cheese flavor and taste. Proteins in the ripening process form many compounds contributing to the taste of cheese. Particulars were described. Milk sugar in Dutch cheeses is completely changed to lactic acid, giving a fresh taste, particularly if pasteurized milk was used. Bacteria can form many other products out of lactic acid; the calcium salt of propionic acid causing the sweet taste of Emmentaler cheese comes from this source. All compounds together form the typical cheese tastes. Knowledge of the taste components and the way they are formed is considered very important for the cheese industry.

A. F. Tamsma

209. Cheese pusher. F. B. Branchfield, Sr., U. S. Patent 2,457,441. 5 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, **617,** 4: 1130. 1948.

An implement for pushing cheese in a cheese vat, consisting of an angular frame and handle

and a screen which may be adjusted to an upright position, is described. R. Whitaker

210. Apparatus for treating cheese. O. F. HANSEL. U. S. Patent 2,455,579. 3 claims. Dec. 7, 1948. Official Gaz. U. S. Pat. Office, **617,** 1: 180. 1948.

Cheese of the Blue or Roquefort type is improved by admitting air to the interior through holes punched uniformly in the cheese by a group of small rods which are forced down through the cheese as it is held firmly between 2 plates so perforated to allow passage of the rods. The rods are lowered and raised automatically by an eccentric operated by a source of power. R. Whitaker

Also see abs. no. 214.

CONDENSED AND DRIED MILK; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

211. Gärungsgetränke aus Pflanzenextrakten und Molke. (Fermented beverages from plant extracts and whey). English summary. M. E. SCHULZ AND K. FACKELMEIER. Die Milchwissenschaft, **3,** 6: 165–174. June, 1948.

Milone is a sweet-sour, slightly alcoholic fermented drink prepared from plant extracts and whey. Whey is fermented to 1% lactic acid and mixed while cold with an equal volume of 3% herb tea. The tannin in the tea is precipitated by the albumin in the whey and the precipitate removed. The liquid is fermented with lactose-fermenting yeasts and sweetened. The maximum alcohol content of the beverage is 0.8%. The herb tea is made up of leaves of linden, hazel nut, billbery and milfoil, together with spicy herbs such as elder blossoms, rosemary linden blossoms and mint. The technical problems involved in the preparation of milone are discussed in detail and illustrations of a processing plant are given. I. Peters

212. Eighty per cent butterfat cream, its production and use. R. J. SPIERS. Abbotts Dairies, Philadelphia, Pa. Ice Cream Trade J., **45,** 1: 44. Jan., 1949.

See abs. no. 87, page A19.

W. H. Martin

213. Treatment of rennet casein for glue manufacturers. A. J. LAWRENCE. Australian J. Dairy Technol., **3,** 3: 96–97. July–Sept., 1948.

Rennet casein showing certain quality defects is unsuited for the manufacture of plastics. It also is unsuited for use in gluemaking. In a search for an additional outlet for such casein, it was found that when rennet casein was treated for 1 hr. with 3 parts by weight of 1% sulfuric acid followed by washing 3 times, filtering and drying at 130° F., a casein showing minimum swelling, good color and an ash content of 2.8% was obtained. Glue prepared from such a product met the requirements of 1,000 lb./in.^{2} breaking strain as prescribed in the "Australian Standard Specification for Joiners' Glue (casein) (1941)". Casein treated similarly with hydrochloric acid and subsequently used in the preparation of glue was not satisfactory. J. C. Olson, Jr.

214. Untersuchungen zur Bewertung der Molke. (The determination of the quality of whey.) English summary. G. DEMMLER AND K. KUMETAT. Die Milchwissenschaft, **3,** 5: 138–142. May, 1948.

In order to determine watering and/or biological deterioration of whey, various methods were used. The ripening of sweet rennet whey by natural fermentation or by addition of pure homofermenting lactic cultures resulted in a lowering of specific gravity, % of total solids and % lactose, thus making these methods unsuitable for the determination of watering of whey. Whey, which is defined by law as "the liquid obtained in cheesemaking after separation of casein and fat by curdling of milk", should contain not less than 5.5% dry matter when made with acid and rennet, and not less than 5.2% when made with acid alone. Thus whey which is in the normal range of acidity but contains less than the prescribed % dry matter should be regarded as inferior and treated accordingly. Proper cooling and/or pasteurization should be practiced in order to maintain the quality of whey. I. Peters

215. Geschichtliches über Molke und Milchzucker. (Historical data in whey and milk sugar.) English summary. E. FUNCK. Die Milchwissenschaft, **3,** 6: 152–160. June 1948.

Documents of the Middle Ages divide milk into butter, cheese and whey. The whey was valued for its nutritional and curative properties. Special attention was given to milk sugar, which was discovered scientifically by Bartoleti in 1633. The name "milk sugar" was used first by Ludavici Testi who used it as a patent name for his medicine against arthritis. In 1711 Fick named the carbohydrate found in milk "milk sugar." Proof that milk sugar is an animal type of sugar was obtained by Scheele in 1780. I. Peters

216. Deformation der Fettkugelchen in Hochkonzentriertem Rahm mit 80 Prozent Fett. (De-

formation of fat globules in highly concentrated cream with 80 per cent fat.) English summary. W. MOHR AND J. WELLM. Die Milchwissenschaft, **3,** 6: 149–152. June, 1948.

Microscopic observations of cream with over 75% fat verified the calculated deformation of fat globules at such high concentrations. The deformation of globules means an increase in potential (surface?) energy and, in turn, lowers the stability of the emulsion. In order to escape the pressure causing deformation, the globules can fuse. The fusing is governed by other factors which are responsible for the stability of the emulsion. The deformation of fat globules in 80% cream may denote the first step in buttermaking by the Alfa process. I. Peters

217. Milk turning machine. J. W. GIBLER. U. S. Patent 2,456,630. 5 claims. Dec. 21, 1948. Official Gaz. U. S. Pat. Office, **617,** 3: 763. 1948.

Evaporated milk in cartons stacked on pallets may be inverted easily by one man with this device. Turning is desirable to eliminate cream separation in the stored product. R. Whitaker

218. Some observations on the bacteriology of condensed milk. H. B. HAWLEY. Henry Edwards and Sons., Ltd., Pipe-Gate, Near Market Drayton. Proc. Soc. Applied Bact., **1946,** 1: 1–5. 1947.

The bacterial count of sweetened condensed milk declined in the course of each day's operation as well as during each weekly period of operation. The high counts occurred immediately after the daily cleaning of the pans and canning machine and after the weekly cleaning of the condensed milk storage vat and pump. These increased counts were considered caused by incomplete cleaning and sterilization of the vacuum pans, packing gland of the pump and the bottom bearing of the agitator in storage vat. The lowering of the count following such periods was caused by the high concentration of solids in the sweetened condensed milk. It was concluded that more attention was needed to constructional design in such plants so that all equipment could be dismantled, cleaned, sterilized and dried.
M. L. Speck

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

219. Thermoduric and thermophilic bacteria in washed milk churns. S. B. THOMAS, L. B. JONES, M. A. BEAVAN, AND B. F. THOMAS. Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., **1946,** 1: 54-55. 1946.

Milk can rinses showed higher counts at 30 than at 37° C., both before and after laboratory pasteurization at 63° C. for 30 min. The can usually had a high thermoduric count. The 627 thermoduric bacteria isolated from yeastrel milk agar plates incubated at 30° C. were classified as follows: microbacetria, 62.0%; aerobic sporeformers, 18.8%; micrococci and sarcina, 7.5%; gram-negative rods, 7.3%; streptococci, 2.2%; yeasts, 1.4%; actinomycetes, 0.8%. Thermophiles were not encountered frequently in the cans, 3.5% showing counts greater than 500,000/ml., while 77% had counts less than 500.
M. L. Speck

220. The reproducibility of dye reduction tests with pasteurized milk. D. W. WATSON, C. J. BESSELL, E. GOLDSCHMIDT, AND J. G. DAVIS. Express Dairy Co., Ltd., 133 Euston Rd., N. W. 1. Proc. Soc. Applied Bact., **1946,** 1: 56–58. 1948.

Resazurin gave somewhat better reproducibility in reduction time than methylene blue on replicate samples of raw and pasteurized milk. Both dyes showed better reproducibility with raw than with pasteurized milk. Also the variation in replicate tubes decreased in samples having shorter reduction times. The samples were incubated until complete reductoin of the dye occurred, and resazurin was reduced more slowly than was methylene blue in both raw and pasteurized milk. M. L. Speck

221. Das Verhalten der Paratyphus-Enteritis-Gruppe in der Molke. (The behavior of the paratyphoid and enteritis groups in whey.) English summary. R. HELM AND W. WEDEMANN. Die Milchwissenschaft, **3,** 5: 133-138. May, 1948.

Samples of rennet and of acid whey were inoculated with pure cultures of the paratyphoid-enteritis group and incubated at room temperature and at 37.5° C. Daily plating on regular agar medium and on Drigalski's medium were made, and the typical colonies obtained examined for motility, gram reaction and agglutination test by appropriate methods. Results of 4 trials with rennet whey showed survival of pathogens up to the 23rd d. in samples held at room temperature, but only up to the 15th d. in those held at 37.5° C., whereas in 7 trials with sour whey no pathogens were isolated after incubation for 7 d. at room temperature and after incubation for 2 d. at 37.5° C. The detrimental effect of acid at higher temperature upon the pathogens is evident. From the health standpoint whey should be pasteurized prior to consumption by humans or animals. I. Peters

222. The critical humidity in the destruction of Streptococcus salivarius by hypochlorite aero-

sols. J. Wolf and A. B. Batchelow. Milton Antiseptic and Deosan, Ltd., John Milton House, Brewery Rd., London, N. 7. Proc. Soc. Applied Bact., **1946,** 1: 59–61. 1946.

At a temperature of 20-25° C., complete destruction of *Streptococcus salivarius* was obtained in 3 min. at a relative humidity of 56% when 1% hypochlorite aerosol was sprayed to give a concentration of hypochlorous acid gas of 0.5 p.p.m. in the atmosphere. With 45% relative humidity at this temperature, 75% of the bacteria remained. Below 20° C., higher relative humidity was required to obtain an equal kill, while above 25° C. less humidity was necessary.

M. L. Speck

223. An improved medium for the enumeration of bacteria in milk and milk products. M. J. Pelczar, Jr., Univ. of Maryland, College Park, and H. D. Vera, Baltimore Biological Laboratory, Baltimore. Milk Plant Monthly, **38,** 1: 30–33. Jan., 1949.

Since the use of the present standard tryptone-glucose-beef extract agar containing milk as a medium for the enumeration of bacteria in milk products offers several disadvantages, the authors propounded a new medium that does not contain meat extract or added skim milk. The composition is as follows: pancreatic digest of casein (Trypticase), 15 g./l.; papaic digest of soy meal (Phytone), 5 g./l.; dextrose, 5 g./l.; sodium chloride, 4 g./l.; cystine, 0.2 g./l.; sodium sulfite, 0.2 g./l.; agar, 15 g./l.; pH, 7.2.

Using standard plating procedures, a series of counts was made on various dairy products. The new medium frequently produced larger colonies than did the standard tryptone-glucose-beef extract agar, yet all counts were comparable. Besides being easily prepared, the new medium, due to its clarity, did not obscure small colonies.

J. A. Meiser, Jr.

224. A method of collecting sterile blood from cattle. F. V. Washko. Purdue Univ., Lafayette, Ind. Am. J. Vet. Research, **9,** 33: 359. Oct., 1948.

A method is described for obtaining blood from the jugular vein of cattle without contamination. The vein is punctured with a sterile 18-gauge needle passed through a 14-gauge needle used to pass through a disinfected skin area.

E. W. Swanson

Also see abs. no. 198, 218.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

225. Method of treating lactalbumin. G. P. Butterfield and L. A. Thorne. (Assigned to American Seal-Kap Corp.) U. S. Patent 2, 457,-642. 2 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, **617,** 4: 1178. 1948.

Dehydrated lactalbumin may be suspended easily in colloidal form in water when made as follows: adjust pH to 7.3 to 8.0, add at 100° F. proteolytic enzymes derived from papain and pancreatic sources, increase temperature to inactivate the enzymes in 1 to 20 min., depending on the strength of the enzymes, and dry to form a stable powder. R. Whitaker

226. De voorgestelde normalisatie der methoden ter bepaling van de Zuurheidsgraad van melk. (The standardization of the method for the determination of the acidity of milk) English summary. C. I. Kruisheer. Rykszuivelstation, Leiden, Holland. Netherlands Milk Dairy J., **2,** 3: 127-136. July-Sept., 1948.

The principal methods of determining milk acidity (Soxhlet-Henkel, Dornic, Thörner, Mann and Van Norman) use different quantities of milk, sometimes diluted with water. Other differences are the quantity of indicator, the concentration of alkali and the way of expressing results.

Variation in alkali concentration and dilution of the milk can be considered the same factor. A slight amount of water makes little difference (Soxhlet-Henkel, Dornic, Mann), large amounts cause too low and unpredictable values (Thörner, Van Norman) by hydrolysis of calcium caseinate.

Variation in the quantity of indicator causes large differences, especially if small amounts are used. It is best to use a rather large quantity, allowing accurate measurement and giving an easy color change with easily observable end point.

The best way of expressing results would be in decimal units; the number of 0.1 $cm.^3$ of 0.1 N alkali for 10 $cm.^3$ of milk was proposed. By using 0.4 $cm.^3$ of 2% phenolphthalein solution and titrating without dilution, the most desirable form of acidity determination suitable for standardization was obtained. A. F. Tamsma

227. Variations in milk and their effects on processing. Part I. How variations in the fat and total-solids affect the properties of milk. W. I. Tretsven. New Mexico A. and M. College, State College. Milk Plant Monthly, **37,** 11: 33. Nov., 1948 .

The physical and chemical properties of milk are altered considerably by variation in milk composition. Since the total solids content of milk is relatively small in relation to the moisture content, any change in composition would exert the most pronounced effect on the fat, pro-

tein, sugar and mineral portion of the milk. Milk fat, which is comprised of many fatty acids, shows pronounced variations when analyzed chemically. This fact accounts for fats with high or low melting points, and this in turn alters many plant procedures, such as churning time and temperature, homogenization and separation. Variations in protein content of milk, coupled with the hydrophilic nature of this constituent, exert a pronounced influence on the physical and chemical properties of milk; processing may be altered to a considerable degree.

J. A. Meiser, Jr.

228. Variations in milk and their effects on processing. Part 2. How variations in the proteins, salts, and the other components affect the properties of milk. W. I. TRETSVEN. New Mexico A. and M. College, State College. Milk Plant Monthly, **37,** 12: 37, 73. Dec., 1948.

Variations in the protein constituents of milk are not uncommon. Colloidal casein affects the color, viscosity, density and stability of milk, whereas the extremely hydrophilic albumin and globulin influence the creaming properties of milk, especially when exposed to pasteurization temperatures. Variations in lactose composition are not uncommon in mastitis. Also, this component, due to its ease of crystallization, governs ice cream composition to a great degree. Although little is known about the true variations in the salt composition of milk, these changes definitely influence the heat stability and coagulating properties of milk. Increased knowledge of milk components will affect future methods and processing procedures in the dairy industry.

J. A. Meiser, Jr.

Also see abs. no. 213.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

229. Pasteurizing system. R. E. OLSON. (Assigned to Taylor Instrument Co.) U. S. Patent 2,455,605. 3 claims. Dec. 7, 1948. Official Gaz. U. S. Pat. Office, **617,** 1: 187. 1948.

The novel feature of this HTST pasteurizing system is a T-shaped fitting having its opposite ends sealed with flexible diaphragms and connected, one with unpasteurized milk as it flows through the heater section and the other with the pasteurized milk as it flows through the cooler. Should the pressure on the pasteurized milk fall below that of the unpasteurized milk, the diaphragms are depressed in such a manner to operate an electrical switch which stops the unpasteurized milk pump, thus preventing any possible leakage of raw to pasteurized milk.

R. Whitaker

230. Method of sterilizing and preserving. A. BRASCH. (Assigned to Electronized Chemicals Corp.) U. S. Patent 2,456,909. 17 claims. Dec. 21, 1948. Official Gaz. U. S. Pat. Office, **617,** 3: 833. 1948.

Foodstuffs, including dairy products, may be sterilized without affecting flavor and physical properties, by bombardment with high speed electrons of a velocity equivalent to at least 1 million volts for 0.0001 sec. R. Whitaker

231. Manufacture of ice cream. Refrigerating Engineering Application Data 41. CHESTER J. BELL, Chairman ASRE Technical Committee on Dairies and Allied Industries. Refrig. Eng., **55:** 1. Jan., 1948.

A basic presentation of the varied items of ice cream manufacture covering make-up of ice cream mix, processing ice cream mix, freezing and hardening ice cream is given. Included is a representative flow diagram of ice cream plant equipment employing the continuous freezer, along with operating features and refrigeration applications. Detailed calculations of refrigeration requirements are presented to illustrate refrigeration loads involved in mix cooling, mix freezing and ice cream hardening. L. M. Dorsey

232. Measurement of holding time of high-temperature, short-time pasteurizers. W. JORDAN AND R. F. HOLLAND. Cornell Univ., Ithaca, N. Y. Milk Plant Monthly, **37,** 11: 85. Nov., 1948

Since the margin of safety is exceedingly small in the high-temperature, short-time pasteurizers, accurate timing of the holding period is essential. Injecting varying concentrations of salt (NaCl) into the holding tube and measuring by electrical conductivity the length of time necessary for the salt to be discharged at the far end of the tube showed a saturated solution of salt to be the most satisfactory for determining the holding period. It also was observed that the injected salt solution should be directed across the flow since comparisons of "against-the-flow" to "with-the-flow" produced 0.7 sec. differences in the holding period as measured by this method. Spray-type nozzles were best for injecting the saturated salt solution. J. A. Meiser, Jr.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

233. Important factors in your operating costs. L. C. THOMSEN. Univ. of Wisconsin. Natl. Butter Cheese J., **40,** 1: 74–76. Jan., 1949.

Dairy plant operators must watch operating costs, particularly during period of general de-

pression or inflation. With few exceptions, the dairy industry returns a greater portion of the consumer's dollar to the primary producer than any other industry. To maintain this favorable position great interest is shown in the importance of processing costs.

In a simple method of expressing operating costs, these are classified broadly into the following groups: personal services; material costs; services; general expense; property expense; profit before dividends and interest; taxes. In the dairy industry wages paid to labor have increased approximately 81% since 1939 with little increase in output per individual, indicating a similar increase in labor costs per unit output. A greater output per worker is advocated to compensate for this rise. The minimum figure for profit for the dairy industry should be 2.1% of gross income or 12% of net worth. The ratio of net worth to gross income serves as a means of evaluation of a dairy business. This ratio should be approximately 1:5.7. The plant operator should departmentalize his cost figures to gain the utmost advantage from them. H. E. Calbert

234. Good will, the hidden treasure in business. Fred Merish. Milk Plant Monthly, **37,** 12: 60–62. Dec., 1948.

A discussion of factors necessary to purchase, compute and maintain good will is presented. Two methods for estimating the good will value of a dairy enterprise are presented together with illustrations of the application of the methods.
J. A. Meiser, Jr.

235. Problems in the operation of city milk plants. L. W. Hoyt. Detroit Creamery Co., Detroit, Mich. Milk Plant Monthly, **37,** 12: 104–106. Dec., 1948.

The task of directing and coordinating milk processing today involves nothing more than utilizing sound fundamental plant-operating procedures. This includes such basic items as: (a) regulated receiving, (b) proper production accounting, (c) adequate inventories, (d) utility checks, (e) product waste control, (f) minimized bottle breakage, (g) good housekeeping, (h) product control and (i) proper plant maintenance.

The future necessitates solving additional problems such as: (a) conversion to new type equipment, (b) increasing speed of plant operations, (c) designing more economical methods of warehousing and (d) regulating the quality and appearance of the product to meet new standards.
J. A. Meiser, Jr.

236. Medosweet emphasizes dealers. E. R. Lucas. Ice Cream Trade J., **44,** 12: 44, 45. Dec., 1948.

This Tacoma, Washington, company attributes the steady and consistent growth in consumer acceptance of its product to quality of product, dealer education and point of sale promotion. The dealer education program consists of showing him how to dip ice cream correctly, how to make a profit by correct dipping and suggestive selling and the possibilities of the ice cream department in necessary sales in other departments.
W. H. Martin

237. What consumers think. Anonymous. Ice Cream Trade J., **44,** 12: 41, 79–81. Dec., 1948.

The General Ice Cream Corp. of Schenectady, N. Y., assisted by 85 drug store owners in Connecticut, has made a marketing research study involving 9,290 consumers. For a big majority, opening and closing hours were satisfactory and most of the customers said the packaged ice cream was kept hard enough to reach home in a satisfactory condition Many (31.5%) said that their home refrigerator would not keep ice cream satisfactorily. Fountain service was good, according to 84.5% of the replies and only 2.8% thought it was poor. A majority (54.1%) considered 20¢ a fair price for a good soda and 55.1% thought 25¢ was a fair price for a good sundae. On the question of size of serving, 60.8% indicated a preference for a larger portion priced at 30¢ to a smaller one at 20¢. The information obtained was supplied to dealers with comments on how to increase sales. W. H. Martin

238. Safeguard your markets. E. J. Mather. National Dairy Products Corp. Ice Cream Trade J., **44,** 12: 50, 52, 63, 65, 88–92. Dec., 1948.

The author recommends that manufacturers teach their dealers to price for profit dollars instead of for profit percentages as a means of maintaining and increasing per capita consumption of ice cream. W. H. Martin

239. Collection time is selling time. R. W. Engle. Engle Dairy, Philadelphia, Pa. Milk Plant Monthly, **38,** 1: 34–35. Jan., 1949.

A comparison of every-other-week collection with weekly collection is made. The author maintains that weekly collection allows routemen to contact working housewives more often, thus promoting future sales through personal contact. Every-other-week collection, he maintains, builds up sales resistance since mounting milk bills tend to force the customer to eliminate the extras.
J. A. Meiser, Jr.

240. A customer solicitation program. Ross Miller. Milk Plant Monthly, **38,** 1: 46–47. Jan., 1949.

This program requires that routemen submit a weekly list of 12 prospects or potential customers to the plant office. A 12-page booklet describing the operations of the company plus a form letter is sent to the prospective customer followed by a free bottle of milk. The driver then contacts the recipient 2 or 3 days later. This practice of solicitation has been very successful in adding new customers. J. A. Meiser, Jr.

241. A year-round incentive plan. Ted Knight. Milk Plant Monthly, **38,** 1: 60, 65. Jan., 1949.

For each new customer picked up and retained for a 30-day period, the routeman receives a bonus of 50 cents per qt. for the month's average daily delivery to the new customer plus the usual dairy products percentage. Wholesale routemen receive $2.40 for each case of milk ordered by a new customer for a 30-day period.

J. A. Meiser, Jr.

242. Don't live off your reserves. H. J. Ashe. Milk Plant Monthly, **38,** 1: 48, 50. Jan., 1949.

To maintain equipment and rolling stock it is extremely important that a depreciation reserve be maintained religiously; otherwise, earnings will be reduced because of antiquated equipment or accumulated replacement costs.

J. A. Meiser, Jr.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

243. Fermentation ability of ingesta from normal and atonic bovine rumens. E. C. Stone. State College of Washington, Pullman. Am. J. Vet. Research, **10,** 34: 26–29. Jan., 1949.

Rumen ingesta from normal rumen-fistulated cows produced from 112 to 250 ml. gas when incubated for 4 hr., compared to 4.5 to 59 ml. from cows with atonic rumens operated on for removal of foreign bodies. Experimental traumatic reticulitis or fasting 48 hr. produced the typical atonic rumen reaction. Volatile acidity of normal rumen ingesta was 84 to 167 ml. 0.1 N, while from atonic rumen ingesta it was 24 to 88 ml. 0.1 N. Volatile acidity varied with time of feeding. E. W. Swanson

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

244. A summary, breeding failure survey in Washington. R. E. Erb and A. O. Shaw. State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 40. 1948.

Analysis of 15 dairy herds which had exhibited a high incidence of breeding difficulties revealed that the following were associated with low breeding efficiency of the cows studied: poor nutrition, disease of the reproductive tract, short interval between parturition and insemination, irregularity of intervals between inseminations and low breeding efficiency of the sire. There was an indication that breeding difficulties were more frequent among cows born in the spring of the year than among those born at other seasons. No significant difference was found in the rate of conception following artificial insemination as compared to natural breeding.

N. L. Jacobson

245. Results of the proved sire program in the Holstein herd at the University of Idaho. D. L. Fourt and R. H. Ross. Idaho Agr. Expt. Sta., Moscow. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 58. 1948.

The continuous selection of sires on the basis of the type and production of their daughters resulted in an increase in the average butterfat production and an improvement in the type of the Holstein herd at the University of Idaho.

N. L. Jacobson

246. Factors influencing the methods of bovine semen evaluation. B. A. Ash and R. E. Erb. State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 76. 1948.

Studies of undiluted bovine semen revealed that the survival time of sperm at 45° C. was greatest at concentration levels of 800,000 to 1,000,000 sperm per cubic mm. The survival of undiluted semen in storage at 40° F. was correlated positively with survival time at 45 °C. and with the ascorbic acid levels of whole semen. A significant positive correlation was found between methylene blue reduction time and ascorbic acid values of semen. N. L. Jacobson

Also see abs. no. 195.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

247. Milk pail cover. A. C. Weiby. (Assigned to Solar Corp.) U. S. Patent 2,460,049. 8 claims. Jan. 25, 1949. Official Gaz. U. S. Pat. Office, **618,** 4: 1129. 1949.

A milk pail cover having a pair of tubes for attaching the teat cup hoses and a pair for attaching to suction and pulsator is described. One control handle closes the latter and opens the former to permit removal of the cover from the pail for emptying. Turning the handle in the opposite direction reverses the valves and places the equipment in operation. R. Whitaker

248. Pressure Releaser Milker. F. G. HODSDON. (Assigned to International Harvester Co.) U. S. Patent 2,458,779. 7 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, **618,** 2: 543. 1949.

The vacuum pulsations on a milker are passed through a sealed can. The milk from the teat cups enters this can and then is removed automatically through an outlet attached to a container within the can. Removal is accomplished by having a novel valve in the small container; this valve opens and fills the container when vacuum is applied and then closes and forces the milk out the outlet when air pressure is applied.

R. Whitaker

249. Breaking lead halter for cattle. J. G. LAWRENCE. U. S. Patent 2,457,246. 2 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, **617,** 4: 1081. 1948.

A halter suitable for leading cattle consists of a loop which encircles the animal's nose and is held in place by a head strap. The loop is made of 2 curved members, hinged on top and bolted together on the bottom, each member carrying a short post and receiving ring on the top.

R. Whitaker

250. Cattle restraining device. H. FLOHR, JR. U. S. Patent 2,458,582. 4 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, **618,** 2: 492. 1949.

This device is placed across a gateway where the animals may enter it one at a time. The animal within the device is restrained between two side members which are drawn together and clamped. The holder then may be swung to a horizontal position to facilitate surgical work, dehorning or branding. R. Whitaker

251. Bull halter and controller. H. MASBRUCH. (Assigned to Russell Mfg. Co.) U. S. Patent 2,458,717. 2 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, **618,** 2: 526. 1949.

The novel feature of this halter is a pair of blinders which cover the eyes of the animal allowing forward sight if the head is held high and preventing fordward sight if the head is lowered for charging. R. Whitaker

252. A study of calf-raising facilities in the State of Washington. G. W. SCOTT, JR., R. E. ERB, A. O. SHAW, R. O. GILDEN AND W. E. MATSON. State College of Wash., Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 70. 1948.

Calves were raised satisfactorily in an open pen-type shed during the winter of 1947–48. Radiant heating units were unsatisfactory as substitutes for straw bedding for calves.

Facilities available for calf raising in the state of Washington are summarized.

N. L. Jacobson

ICE CREAM

C. D. DAHLE, SECTION EDITOR

253. Ice cream packer. L. J. GILCREST.. U. S. Patent 2,457,541. 2 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, **617,** 4: 1154. 1948.

A cylindrically-shaped utensil for packing retail packages of ice cream is described. It is equipped with a handle to push the device down into ice cream and a piston which ejects the ice cream from the cylinder when pressed down by a handle which is attached to the piston.

R. Whitaker

254. Arden's diced cream. ANONYMOUS. Ice Cream Trade J., **45,** 1: 36. Jan., 1949.

A new package for individual servings of ice cream and a filling machine has been developed. The carton, made of light, waxed paperboard, has a capacity of 3.5 fl. oz. and forms a 2-in. cube of ice cream. The cartons are set up, filled with semi-frozen ice cream, closed by the machine and moved on a conveyor to a hardening room. Freezing is completed in 35 to 40 min., after which they are packed by hand in a heavy chipboard box for distribution to stores. New glassware also has been developed for use in dispensing this new form of ice cream. Quicker, more sanitary dispensing without dipping loss are some of the advantages claimed for the new package.

W. H. Martin

255. 1948 gallonage expected to top 550 million gallons. ANONYMOUS. Ice Cream Trade J., **44,** 12: 42, 68. Dec., 1948.

On the basis of U.S.D.A. reports for the first 10 mo. of 1948, which indicate a drop of 11% from the same period in 1947, the gallonage for the entire year will be about 550 million.

W. H. Martin

Also see abs. no. 231, 236, 237, 238.

MILK

P. H. TRACY, SECTION EDITOR

256. Cream dispenser. J. P. JONES. (Assigned to Dairy Specialties Co.) U. S. Patent 2,459,130. 6 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, **618,** 2: 633. 1949.

Two holes are provided in the side of conven-

tional paper milk bottles, one at the lower cream level and one at the upper cream level. The holes are closed by a flap which may be swung downward to uncover the upper or upper and lower hole, for removal of milk or cream.

R. Whitaker

257. Milk container. J. P. JONES. (Assigned to Dairy Specialties, Inc.) U. S. Patent 2,457,822. 4 claims. Jan. 4, 1949. Official Gaz. U. S. Pat. Office, **618,** 1: 140. 1949.

A square-shaped paper milk bottle is described which employs a perforated shelf located at the level of the bottom of the cream line which facilitates removal of the cream through a wall opening sealed with a partially severed closure tongue.

R. Whitaker

258. Some aspects of pasteurization in a market milk plant. A. G. F. ITZERODT. Australian J. Dairy Technol. **3,** 3: 91–95. July–Sept., 1948.

The effects of low-temperature, holding (LTH) and high-temperature, short-time (HTST) methods of pasteurizing milk on cream line and on bacterial destruction were compared. The temperature and time relationship was 145° F. for 30 min. for the LTH method and 162° F. for approximately 32 sec. for the HTST method. No serious reduction in cream line was observed using the LTH method unless the temperature exceeded 148° F. or the holding period exceeded 45 min. The HTST method likewise had little effect on cream line when measured immediately after pasteurization and cooling. Temperatures above 162° F. (presumably with 32 sec. holding) materially reduced the cream line. Storage of milk after HTST pasteurization for 5, 8.5 and 16 hr., with subsequent agitation prior to bottling resulted in a cream line reduction of 25, 36 and 45 to 50%, respectively. With LTH pasteurization, cream line reduction after similar treatment did not exceed 10%. Plate counts of pasteurized milk, using Difco tryptone-glucose-extract medium with incubation at 37° C. for 48 hr., were obtained at intervals during 1 mo. before and 1 mo. after a change from LTH to HTST pasteurization. During the period of LTH pasteurization, bacterial counts were somewhat lower than those obtained during the HTST pasteurization period. Direct comparisons between the two systems of pasteurization using a split lot of the same milk were not made. Counts obtained after "laboratory pasteurization" agreed with counts obtained after LTH pasteurization but not with counts after HTST pasteurization. In accounting for the differences in bacterial destruction by the two methods of pasteurization, the suggestion was made that the physiological condition of the cells may be important, in that mature or aged cells may show a greater degree of heat tolerance to the HTST process than to the LTH process.

J. C. Olson, Jr.

Also see abs. no. 220, 223, 227, 228, 229, 232, 235, 239, 240, 241, 259, 260.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

259. Translating dietary allowances into foods and diets. L. A. MAYNARD. Cornell Univ., Ithaca, N. Y. J. Am. Dietetic Assoc., **25,** 1: 18–20. Jan., 1949.

The most significant of the changes in the new Recommended Daily Allowances is the increased recommendation for Ca in the adult diet. The mere addition of more milk and green leafy vegetables in the diet is difficult, since many of the food habits of adults would have to be changed, milk supplies in many localities are inadequate to meet present demands and, in some cases, the incomes of families do not permit them to buy more milk. A desirable plan would be to increase the milk supply, not so much through agricultural production as through more efficient use of present supplies, better distribution and consumer education. Low income groups could be aided by extending public funds, now used to care for the sick, for use in preventing illness by providing adequate diets for those unable to obtain them. In addition to providing more of the superior foods, such as milk, dietary improvement can be brought about through an improvement in the nutritional quality of foods now produced, processed and marketed. The establishment of nutritional standards for foods would involve too many difficulties, but the consideration of such an idea would bring before the public the need for superior nutritional quality of available food supplies.

Elizabeth K. Wheeler

260. Milk in human nutrition. C. P. SEGARD, M. C. Wisconsin Alumni Research Foundation, Madison. Milk Plant Monthly, **37,** 12: 58–59, 108–109. Dec., 1948.

The author presents a complete discussion of the importance of vitamins, milk solids, minerals and milk sugar in human nutrition. A discussion of the nutritional value of special milk products such as homogenized milk, "Vitamin D Milk" and fat-free milk also are included.

J. A. Meiser, Jr.

261. Increasing the "invisible milk" in the daily food intake. M. B. BALSLEY, Nutrition Consultant. Evaporated Milk Assoc., Chicago,

Ill. J. Am. Dietetic Assoc., **25,** 1: 58–64. Jan., 1949.

Adult diets are in need of revision in view of the present increase in the Recommended Daily Allowances for Ca from 0.8 to 1 g. More milk can be provided by including dishes prepared with milk. There is considerable variation in the amounts of milk used for the same dish in different recipe books. The selection of those recipes using larger quantities of mlik and more concentrated sources, such as dried and evaporated milk, can increase considerably the milk consumption by adults. The inclusion of more cheese and ice cream in the diet is recommended. Additional milk may be used on cereals, in bread-making, on vegetables and in soups, main dishes and desserts. The planning of menus to supply regular amounts of milk in these ways can supplement reduced amounts consumed as a beverage by adults.

Elizabeth K. Wheeler

262. Effect of food supplements on growth, reproduction and lactation. P. B. JUNQUEIRA AND B. S. SCHWEIGERT. Texas A. & M. College, College Station. J. Am. Dietetic Assoc., **25,** 1: 46–49. Jan., 1949.

A study was made of the adequacy, for growth, reproduction and lactation in the rat, of diets composed of basal mixtures of whole milk powder and whole wheat, supplemented with meat, casein, liver extract and other factors. Supplementing the basal diet with 11.4% of canned beef caused improvement in the performance of the rats in each of these respects. When the basal ration contained 16.7% whole milk powder, the addition of beef increased the average weaning weights of the young rats from 26 to 42 g. and the average number of young born to the female from 8 to 11. When whole milk powder composed 33.3% of the basal ration, the weights of the weanlings increased from 39 to 43 g. upon the addition of the beef. There was no further improvement in the number of young produced. Improvement in the reproductive performance of the animals was attributed to the additional protein and possibly other unknown factors provided by the beef. The addition of casein caused improvement as did the beef, but the liver extract appeared to have no effect.

Elizabeth K. Wheeler

263. Technik des Tierversuchs und seine Bedeutung für die Bewertung von Milchprodukten. (The technic of the animal test and its importance for the evaluation of milk products.) English summary. LACTRONE G. M. B. H. Die Milchwissenschaft, **3,** 5: 117–125. May, 1948.

Six groups of 10 albino rats in each group were used in determining availability of riboflavin in dairy products. The basal diet consisted of 24% dry whey, 12% washed casein, 14% butterfat and 50% potato starch. The six diets used were (a) basal diet with dry whey and casein replaced by skim milk (12 ml./animal/d.), (b) basal diet plus fresh whey (12 ml./animal/d.), (c) basal diet only, (d) basal diet with dry whey replaced by roasted whey (held 1.5 hr. at 200° C.), (e) diet *d* plus 5% Fuller's earth adsorbate (prepared from whey and containing primarily vitamins B_1 and B_2), (f) basal diet with dry whey replaced by corresponding amounts of lactose, protein and minerals. Optimum weight gains were obtained with diet *a* followed by *b, c* and *d* in the order named. The negative results obtained with diet *f* were due to the absence of vitamins. The slow gains obtained with *d* and *e* were due to the harmful effect of roasting upon the proteins in the whey. The Fuller's earth adsorbate of fresh whey could be used as vitamin B_2 source by the rats.

Four groups of three guinea pigs in each group were used to determine the vitamin C content in the Fuller's earth adsorbate of fresh whey. The basal diet consisted of 65% ground oats, 30% skim milk powder, 1% butter, 1% yeast, and hay *ad libitum*. The ground oats, milk powder and hay were exposed to 110° C. for 6 hr. to destroy the vitamin C. The four diets used were: (a) basal diet only, (b) basal diet plus 0.5 mg. ascorbic acid/animal/d., (c) basal diet plus 20% Fuller's earth whey adsorbate and (d) basal diet plus 40% Fuller's earth adsorbate. The absence of vitamin C in Fuller's earth adsorbate was established.

I. Peters

264. Uber den biologischen Wert des Eiweisses von Kefirpilzen im Vergleich zum Eiweiss der Milch, verschiedener Hefen und Schimmelpilze, sowie zum Kartoffeleiweiss. (On the biological value of the proteins of kefir fungi in comparison with protein of milk, of various yeasts and molds and of potatoes.) English summary. H. Fink. Die Milchwissenschaft, **3,** 5:125–133. May, 1948.

Albino rats were used in feeding trials to obtain biological values of proteins from yeasts, molds and potatoes and to compare them with milk proteins. The liver symptoms observed in feeding yeast proteins could be corrected in most instances by the addition of 0.2% L-cystine to the diet. By assigning to milk protein a biological value of 100, beer yeast was given 84; straw-prehydrolyzate yeast, 64; beechwood alkaline sulfite yeast, 34; wood sugar yeast, 33; "*Aspergillus orycae*", 44; "Biosyn", 53; and potato protein, 54. The type of yeast and its environment, such as growth medium, oxygen relationship, temperature, rate of growth, etc., are mentioned as possible factors controlling the biological value of the resulting protein.

The biological value of kefir protein was 90% of the value of milk protein and was the highest value obtained in plant proteins. Additions of cystine to kefir protein were unwarranted, since no liver symptoms were produced in 90-d. feeding trials. Possible reasons for the high biological value of kefir protein are: (a) symbiosis of bacteria and yeast in kefir, (b) slow growth without aeration, (c) abundant supply of organic nitrogen and growth factors. I. Peters

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

265. Sulfamethazine blood concentrations in calves. C. R. SHROEDER, M. WELSH, R. L. BURKHART, AND P. H. LANGER. Lederle Laboratories, Pearl River, N. Y. Am. J. Vet. Research, **10,** 34: 63–65. Jan., 1949.

Experiments with 2- to 3-mo. old normal calves showed that sulfamethazine accumulated in the blood on a dosage of 1 g./lb. body weight daily. Optimum dosage was 1 g./lb. on subsequent days. The drug also was administered successfully at the same rate in the milk fed the calf and by subcutaneous injection. No evidence of hematuria, inappetence or blood changes was noted.
E. W. Swanson

266. Studies on vehicles for sustaining penicillin levels in the bovine mammary glands. E. J. FOLEY, A. W. STULTZ, S. W. LEE AND J. V. BYRNE. Wallace Laboratories, Princeton, N. J. Am. J. Vet. Research, **10,** 34: 66–70. Jan., 1949.

The penetration and persistence of single doses of 100,000 units of penicillin in water-in-oil vehicles infused into the udder were studied. Vehicle A, containing mineral oil, water and lanolin derivatives, was slightly superior to an aqueous infusion, retaining 1.5 to 2.0 units of penicillin/ml. of milk at 36 hr., compared to none from the aqueous vehicle. Vehicle B, containing mineral oil, water, lanolin derivatives, propylene glycol and a nonionic wetting agent, persisted in the lactating gland longest, having 0.4 to 4 units of penicillin/ml. of milk at 72 hr. The vehicles were without clinical irritating effects. Dye in aqueous solution and in vehicle B showed in the excised udder that the effect of the water-in-oil emulsion was due to its general diffusion throughout the gland, while the aqueous solution went only slightly beyond the cistern area. E. W. Swanson

267. The effect of lactation upon the ascorbic acid values of whole blood and blood plasma. R. G. THOMAS, R. E. ERB AND A. O. SHAW. State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 62. 1948.

In a study of both lactating and non-lactating cows of the Holstein, Jersey and Guernsey breeds a correlation, which was highly significant statistically, was observed between blood plasma ascorbic acid levels and pounds of milk produced per day. When the data for the dry cows were excluded, only the Holsteins showed a significant correlation between the level of milk production and blood plasma ascorbic acid.
N. L. Jacobson

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

268. Control of insects and rodents in dairy plants. E. M. SEARLS, Sealtest, Inc., New York, N. Y. Milk Plant Monthly, **37,** 11: 83. Nov., 1948.

No rodent-control program will be effective unless rodent-proof barriers are constructed in all the openings leading into the building. Once this has been effected, trapping with a clap-type trap baited with foods that the rats like but don't have access to has been very successful. Suggested baits are bacon rind, smoked fish, raw meat, peanut butter, vegetables, bananas and other fruits. Ten mouse traps and ten rat traps should be sufficient for the average-size plant.

Insect control can be effected best using 5% DDT in an odorless kerosene base. Apply this solution to clean surfaces where the insects go to roost or hide. As kerosene evaporates, it will leave dry DDT crystals which are toxic to insects for long periods. This procedure should be repeated every 3 mo. J. A. Meiser, Jr.

Also see abs. no. 218.

MISCELLANEOUS

269. History of research dealing with problems confronting the dairy industry which have been conducted by the Agricultural Experiment Stations. A. R. CONLEY. Ohio State University. Mimeograph. 219 pp. 1949.

This publication is divided into two parts. The first part lists, by individual experiment stations, those bulletin publications during the interval covered which are concerned with material related directly or indirectly to the different phases of dairying. The second part arranges the publications listed in the first part according to the subject matter treated, utilizing the title of the publication as the basis for indexing.

This is an interesting publication which undoubtedly will be of value to many. However, many stations have published extensively outside

of their bulletin series; at other stations certain workers may have chosen to publish in journal articles. Therefore, because journal articles are not included, this compilation may give considerably less than a true picture of either the program and publications of a particular station or the contributions which an individual has made.

F. E. Nelson

270. Dairy research under the Research and Marketing Act. E. A. Meyer, U.S.D.A. Milk Plant Monthly, **38,** 1: 36, 64. Jan., 1949.

Increased efforts are being made under the Research and Marketing Act to promote the complete utilization of milk and its by-products, including such projects as the use of cheese whey in sherbets as the sole source of milk solids and the use of high grade dry buttermilk solids in ice cream. Economic studies relating to the seasonality of milk prices and the function of cooperatives in the development of milk-pricing plans and methods are being conducted, as are studies of the yields of a unit quantity of milk. In the production field, intensive experiments are being conducted to develop new strains of dairy cattle for hot and humid climates and for owners of small dairy herds. J. A. Meiser, Jr.

271. Dependability of food judges as indicated by an analysis of scores of a food-tasting panel. Andrea Overman, Oregon Agr. Expt. Sta., and Jerome C. R. Li, Oregon State College, Corvallis. Food Research, **13,** 6: 441. Nov.–Dec., 1948.

A method of determining the reliability of flavor judgments by members of a food-tasting panel is described and discussed. In the example presented, ten judges scored ten replicate lots containing five food samples having definite flavor variations. The results were analyzed in 2 ways. The score ranges, the number of duplicated judgments and the absolute deviations from the means revealed some of the more obvious differences among the judges. Analysis of variance was used to measure the consistency and the discriminating abilities of the judges. A high ability to recognize differences in samples, together with a low variability in duplicating judgments, are stated as being the marks of a good judge. F. J. Doan

272. Significance in triangular taste tests. E. B. Roessler, J. Warren and J. F. Guymon. Univ. of Calif., Davis. Food Research, **13,** 6: 503. Nov.–Dec., 1948.

The "triangular" or odd-sample method of distinguishing small differences in flavor between two food samples is described. The samples are presented in groups of three, two in each group being identical. Six orders of presentation are possible: AAB, ABA, BAA, BBA, BAB, ABB. When differences are questionable each taster should be served the samples in all six sequences. The results lend themselves to statistical analysis and a table showing the number of correct answers required to establish significant differentiation is presented. F. J. Doan

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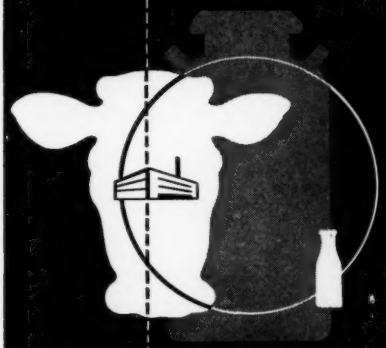

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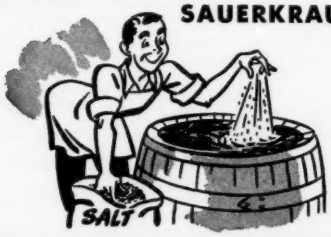

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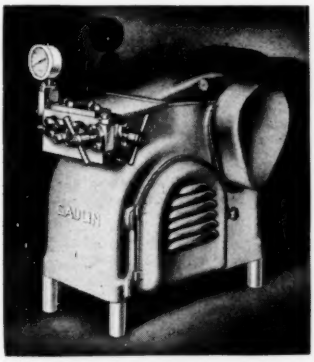

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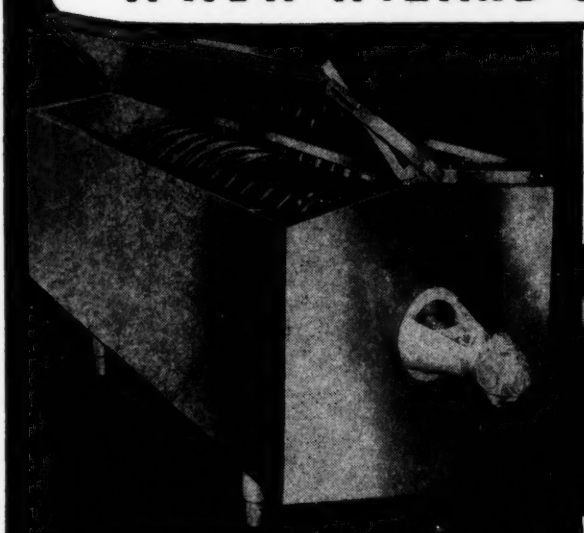

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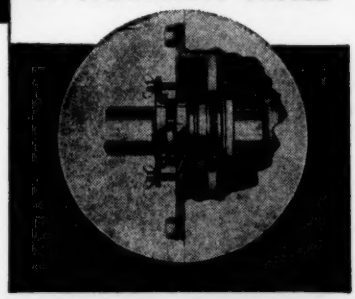

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